

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



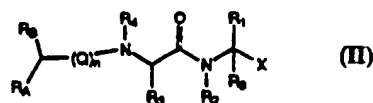
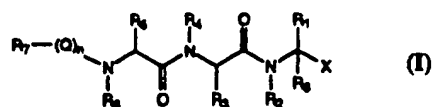
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 5/06, 5/08, 5/02, C07C 237/22, A61K 38/55		A1	(11) International Publication Number: WO 96/20949 (43) International Publication Date: 11 July 1996 (11.07.96)
(21) International Application Number: PCT/US96/00359 (22) International Filing Date: 5 January 1996 (05.01.96) (30) Priority Data: 369,422 6 January 1995 (06.01.95) US		(74) Agent: SEIDMAN, Stephanie; Brown, Martin, Haller & McClain, 1660 Union Street, San Diego, CA 92101-2926 (US). (81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): THE SALK INSTITUTE BIOTECHNOLOGY/INDUSTRIAL ASSOCIATES, INC. [US/US]; Suite 300, 505 Coast Boulevard South, La Jolla, CA 92037 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): MCDONALD, Ian, Alexander [AU/US]; 4722 Shadwell Place, San Diego, CA 92130 (US). ALBRECHT, Elisabeth [US/US]; 10540 Bannister Way, San Diego, CA 92126 (US). MUNOZ, Benito [CA/US]; 6977 Schilling Avenue, San Diego, CA 92126 (US). ROWE, Blake, Alan [US/US]; 4060 Huerfano Avenue #310, San Diego, CA 92117 (US). SIEGEL, Robert, Steven [US/US]; 4360 Huggins Street, San Diego, CA 92122 (US). WAGNER, Steven, Lee [US/US]; 1074 America Way, Del Mar, CA 92014 (US).		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	

(54) Title: PEPTIDE AND PEPTIDE ANALOG PROTEASE INHIBITORS

(57) Abstract

Methods of use of compounds and compounds for the treatment of disorders characterized by the cerebral deposition of amyloid are provided. Among the compounds are those of formulae (I) and (II), in which R₁ is preferably 2-methyl propene, 2-butene, cyclohexyl or cyclohexylmethyl; R₂, R₄, and R₅ are each independently methyl or ethyl; R₃ is preferably *iso*-butyl or phenyl; R₆ is H or methyl; R- (Q)_n is preferably benzyloxycarbonyl or acetyl; Q is preferably -C(O)-; R₈ is preferably *iso*-butyl; R₄ = -(T)_m-(D)_m-R₁, in which T is preferably oxygen or carbon, and D is preferably a mono-unsaturated C₃₋₄ alkenyl; and X is preferably a α -ketoester or α -ketoamide or aldehyde.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

-1-

PEPTIDE AND PEPTIDE ANALOG PROTEASE INHIBITORS

This application is a continuation-in-part of U.S. application Serial No. 08/369,422 to McDonald *et al.*, filed January 6, 1995, entitled *PEPTIDE AND PEPTIDE ANALOG PROTEASE INHIBITORS*. The subject matter of

5 U.S. application Serial No. 08/369,422 is herein incorporated in its entirety by reference.

FIELD OF THE INVENTION

This invention relates to peptidyl compounds useful for a variety of physiological end-use applications. More specifically, di- and tripeptide

10 analogs that are useful in the treatment of neurodegenerative disease states and in the treatment of the degeneration of the neuronal cytoskeleton are provided.

BACKGROUND OF INVENTION

Proteases play a key role in metabolism and in the pathology of

15 numerous disorders. As a consequence, compounds that specifically inhibit protease activity are often therapeutic. For example, renin is an aspartyl protease that cleaves angiotensinogen to angiotensin I. Angiotensin I is hydrolyzed by angiotensin converting enzyme (ACE) to angiotensin II, which is a potent vasoconstrictor and stimulant of aldosterone secretion.

20 Compounds that inhibit the activity of ACE, such as captopril an orally active ACE inhibitor, are therapeutically effective for treating hypertension and congestive heart failure. Renin inhibitors are thought to have similarly beneficial therapeutic activity.

Activated ketone-based inhibitors have been used as inhibitors of

25 different classes of proteases, including serine proteases, aspartyl proteases, cysteine proteases and metalloproteases, because they exist as hydrates in aqueous media and directly serve as transition state analogs and/or react with a nucleophilic residue (such as the serine hydroxyl or cysteine sulfhydryl) to form a reversible hemiacetal-type intermediate. For example,

30 phenylalkyl ketones are potent and competitive reversible inhibitors of inter-

leukin 1- β converting enzyme (ICE) and thus, are thought to have therapeutic use for treatment of certain chronic inflammatory disease states. The precursor alcohols for these ketones have been reported to have substantially reduced protease activity compared to the corresponding ketone [see, e.g., Patel *et al.* (1988) *Tetrahed. Letts.* 29:4665-4668; Patel *et al.* (1993) *J. Med. Chem.* 36:24310-2447].

Certain trifluoromethyl ketones are inhibitors of, for example, human leukocyte elastase (HLE), which is a serine protease. This enzyme has been implicated as a pathogenic agent in a variety of disorders, including pulmonary emphysema, rheumatoid arthritis, adult respiratory distress syndrome (ARDS), glomerulonephritis and cystic fibrosis [see, e.g., Skiles *et al.* (1992) *J. Med. Chem.* 35:641-662; Angelastro *et al.* (1994) *J. Med. Chem.* 37:4538-4554].

Proteases are also implicated in diseases, such as Alzheimer's Disease (AD), that are characterized by the accumulation of amyloid plaques. Amyloidogenic A β peptides (A β) are the principle component of the amyloid plaques that accumulate intracellularly and extracellularly in the neuritic plaques in the brain in AD. A β is an ~4.0 kDa protein, about 39-43 amino acids long, that is derived from a C-terminal region of amyloid precursor protein (APP). APP is a membrane-spanning glycoprotein that, in one processing pathway, is cleaved inside the A β domain to produce α -sAPP, a nonamyloidogenic secreted form of APP. Formation of α -sAPP precludes formation of A β . It has been proposed that A β is generated by virtue of a different processing pathway, so that compounds that inhibit the activity of enzymes responsible for A β production are being sought [see, e.g., Wagner *et al.* *Biotech. Report* (1994/1995), pp. 106-107; and Selkoe (1993) *TINS* 16:403-409].

Because proteases are implicated in numerous disorders, there is a need to develop potent and specific inhibitors of these enzymes. Therefore, it is an object herein to provide methods of treating disorders in which

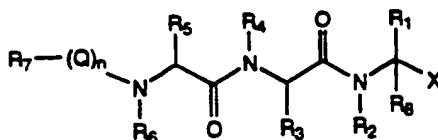
-3-

protease activity plays a pathological role. It is also an object herein to provide protease inhibitors.

SUMMARY OF THE INVENTION

Di- and tri-peptide compounds and methods of treating certain disorders, particularly cognitive disorders, are provided. Methods of identifying, isolating, and inhibiting proteases are also provided. Further provided are methods of identifying protease inhibitors. The methods use compounds having formulae:

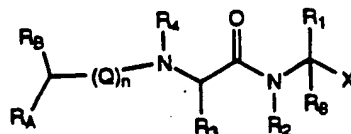
10



15

I

20



25

II

30

or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof in which:

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi) or (vii) as follows:

35

(i) R_1 , R_3 , R_5 , and R_8 , are each independently selected from a side chain of a naturally occurring α -amino acid, H, alkyl, preferably lower (C_{1-6}) alkyl, alkenyl, preferably C_{2-10} alkenyl, alkynyl, preferably C_{2-6} alkynyl, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl,

heteroaralkyl, heteroaralkenyl, Y-substituted aryl, aralkyl, aralkenyl, aralkynyl, and Z-substituted heteroaryl, heteroaralkyl, heteroaralkenyl, in which Y is selected from halogen, lower alkyl, alkoxy, OH, haloalkyl, preferably CF₃, NO₂, nitrile, S-alkyl, phenyl, and -NRR', R and R' are independently selected from H or alkyl, preferably lower alkyl, OH and halo-lower alkyl, particularly CF₃, Z is lower alkyl, preferably C₁₋₄ alkyl, or halo lower alkyl, preferably C₁₋₄ haloalkyl, more preferably CF₃;

R₂, R₄, R₆, and R₈ are each independently selected from among H and lower alkyl, preferably C₁₋₄ alkyl;

R₇ is selected from among C₁₋₆ alkyl, aryl, alkenyl, 9-fluorenyl, aralkyl, aralkenyl, aralkynyl the aryl groups are unsubstituted or are substituted with Z;

Q is selected from among -C(O)-, -O-C(O)-, -C(O)O-, -S(O)₂- and HN-C(O)-;

n is zero or one;

R_A is -(T)_m-(D)_m-R₁ in which T is O or NH, and D is C₁₋₄ alkyl or C₂₋₄ alkene; and m is zero or one;

X is selected from -(CH₂)_rC(O)H, -(CH₂)_rC(O)haloalkyl, -(CH₂)_rC(O)(CH₂)_rCHN₂, -C(CH₂)_r(O)C(CH₂)_r(O)OR_D, -(CH₂)_rC(O)(CH₂)_rC(O)NR_DR_E, -(CH₂)_rC≡N, -(CH₂)_rC(OH)(CH₂)_rC(O)U, -(CH₂)_rC(OH)CH₂C(O)U, -(CH₂)_rC(O)W and -(CH₂)_rC(O)CH₂W, in which: R_D and R_E are independently selected from among H, alkyl, preferably lower alkyl, more preferably C₁₋₄ alkyl, phenyl, benzyl, and phenethyl; U is -OR_D or -NR_DR_E, and W is -OR_D, -SR_D, and -NR_DR_E, or heterocyclic moiety, preferably containing 4-6, more preferably 5 or 6 members in the ring, and preferably one or two heteroatoms, selected from O, S, or N, in the ring, and r is 0-5, preferably 0-3, more preferably 0 or 1, most preferably 0; or

-5-

(ii) $R_1, R_2, R_3, R_4, R_5, R_6$, and X are selected as in (i),

(iv)(a-c) with R_8 being H or (v)(a-c);

V is OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH;

n is zero; and

R_6 and R_7 are selected so that with the atoms to which each is attached they form a heterocyclic moiety, which:

(a) contains from 3 to 21 members and one or two or more fused rings, each ring containing preferably 3 to 7, more preferably 4 to 6, members, and is preferably morpholino, thiomorpholino, pyrrolidinyl, V -substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl, or a reduced isoquinoline, preferably 1,2,3,4-tetrahydroisoquinoline;

(b) does not contain adjacent heteroatoms;

(c) is unsubstituted or substituted with one or more substituents selected from Y , more preferably from V , and most preferably selected from among OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH; or

(iii) $R_1, R_2, R_3, R_4, R_5, R_6$, and X are selected as in (i);

V is as defined in (ii);

Q is $C(O)$;

n is one; and

R_6 and R_7 are each independently selected as follows:

(a) from carbonyl ($C=O$), phenyl, a heteroatom, lower alkyl, preferably C_{1-3} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-3} alkyl, and

(b) each is unsubstituted or substituted with Y , preferably with V , and

(c) together with the atoms to which they are attached form a cyclic moiety, preferably a 4-6 membered cyclic or 8-12 membered bicyclic moiety, and

(d) R_6 and R_7 are selected with the proviso that when two or more heteroatoms are present there is a carbon atom between the heteroatoms; and

(e) the cyclic moiety is preferably succinimide, phthalimide or maleimide; or

(iv) R_3 , R_4 , R_5 , R_6 , R_7 , R_A , R_B , Q, X and n are as defined in any of (i)-(iii) or (v)-(vii),

V is as defined in (ii);

R_8 is H; and

R_1 and R_2 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_1 and R_2 are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

(v) R_1 , R_2 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X, Q and n are as defined in any of (i)-(iv) or (vi)-(vii);

V is as defined in (ii);

R_3 and R_4 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the

-7-

proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) is unsubstituted or substituted with Y, preferably with V, and

5

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl;

10

(vi) $R_1, R_2, R_3, R_4, R_7, R_8, Q, X$ and n are as defined in any of (i), (iv)(a-c) with R_8 being H or (v)(a-c);

V is as defined in (ii);

R_5 and R_6 are each independently selected as follows:

15

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_5 and R_6 are unsubstituted or substituted with Y, preferably with V, and

20

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

25

(vii) $R_1, R_2, R_3, R_4, R_6, R_8$, and X are selected as in (i)

(iv)(a-c) with R_8 being H or (v)(a-c);

V is as defined in (ii);

n is zero; and

R_5 and R_7 are each independently selected as follows:

-8-

(a) from lower alkyl, preferably C₁₋₄ alkyl, or lower alkyl linked to a heteroatom, preferably C₁₋₄ alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

5 (b) R₅ and R₇ are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl;

10

in all instances, unless specified, the carbon chains, which may be straight or branched, contain from 1 to about 12 carbons preferably 1 to 6, and most preferably 4-6 carbons, and the cyclic moieties preferably contain one ring or two fused rings with from 3 to 16 atoms, preferably 4 to 12, with 4 to 6 in each ring, in the ring structures.

15

Unless otherwise stated, the α -amino acids of the compounds of formulae I and II are preferably in their L-configuration. In their preferred configuration with reference to a particular compound, R₁ is S, R₃ is S, and R₅ is R/S. Also, a compound of these formulae may be in free form, e.g., an amphoteric form, or a salt form, e.g., an acid addition or an anionic salt. A compound may be converted to its salt or base form in an art-known manner, one from another. Preferred salts are trifluoroacetate, hydrochloride, sodium, potassium or ammonium salts, although the scope of the salts embraced is not limited thereto, the scope being extended to include all of those salts known to be used in the art of chemistry.

20

25

Compounds are also provided herein. These compounds may be used in the methods. The compounds have formulae I or II as defined above, but with the proviso that, when the compounds have formula (I): (1) at least one of the amino acid residues in the resulting tri-peptide is a non-naturally-

30

-9-

occurring α -amino acid or at least one of the R_1 , R_3 and R_6 is not a side chain of a naturally-occurring amino acid; and (2) when X is an aldehyde, the non-naturally occurring amino acid (or side chain thereof) is other than norleucine or norvaline, and when the compounds have formula (II) and X is an aldehyde, R_1 cannot be the side chain of norleucine or norvaline.

In some embodiments the compounds of formula (II) are also selected such that: (1) at least one of the amino acid residues in the resulting di-peptide is a non-naturally-occurring α -amino acid or at least one of the R_1 and R_3 is not a side chain of a naturally-occurring amino acid; and (2) when X is an aldehyde, the non-naturally occurring amino acid (or side chain thereof) is other than norleucine or norvaline.

In certain preferred embodiments, the compounds have formulae I or II, particularly formula I, as defined above, but with the proviso that: (1) at least one of the amino acid residues in the resulting di- or tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R_1 , R_3 and R_6 is not a side chain of a naturally-occurring amino acid; and (2) when R_1 is the side chain from a non-naturally occurring amino acid, it is not the side chain of norleucine or norvaline.

In other preferred embodiments, the compounds have formulae I or II, particularly when the compounds have formula I, as defined above, but with the proviso that: (1) at least one of the amino acid residues in the resulting di- or tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R_1 , R_3 and R_6 is not a side chain of a naturally-occurring amino acid; and (2) none of R_1 , R_3 and R_6 is the side chain of norleucine or norvaline.

The compounds provided herein are preferred for use in the methods, particularly the methods of treatment of cognitive disorders.

Pharmaceutical compositions containing a compound of formulae (I) and (II) are provided. In particular, pharmaceutical compositions formulated for single dosage administration are provided.

Methods of treatment of diseases, particularly cognitive disorders are provided and are effected by administering an effective amount of the pharmaceutical compositions. In particular, methods of treating a patient suffering from a neurodegenerative disease selected from among Alzheimer's disease, cognition deficits, Down's Syndrome, Parkinson's disease, cerebral hemorrhage with amyloidosis, dementia pugilistica, head trauma and any disorder characterized by an accumulation of plaques in the brain, by administering to the patient a therapeutically effective amount of a compound of formulae (I) and (II) or compounds of formulae (I) and (II) in which R_1 , R_3 , R_5 and R_6 can all be side chains of naturally-occurring amino acids are provided.

Methods of treating a patient suffering from a disease state characterized by the degeneration of the cytoskeleton arising from a thrombolytic or hemorrhagic stroke by administering a therapeutically effective amount of a compound provided herein, particularly a compound of formulae (I) and (II), are also provided.

Assays designed to detect compounds that modulate the processing of APP and other related proteins are also provided. In particular, assays that detect a relative decrease in the amount of amyloidogenic $A\beta$ peptide produced by cultured cells that express APP, such as cultured human glioblastoma cell lines that have been transfected with DNA encoding either a wild-type 695 amino acid isoform of APP or a mutein of APP are provided. A positive in vitro assay occurs when: (1) there is a decrease in the -4-kDa amyloid β -protein ($A\beta$) in the medium relative to control cultures; and/or (2) the relative amount of soluble APP (referred to as total sAPP) in the medium increases (e.g., relative to the amount of total sAPP in medium from appropriate control cells such as cells not exposed to test compound); and/or (3) there is a decrease in the amount of C-terminal fragments of APP larger than 9 kDa in the cell lysate as a result of differential processing;

and/or (4) there is an increase in the amount of α -sAPP in the medium relative to control cultures.

Methods of detecting markers indicative of neurodegenerative disorders characterized by deposition of cerebral amyloid by detecting a decrease in the ratio of α -sAPP to total sAPP or a decrease in the amount of α -sAPP in a sample of CSF compared to such ratio or amount in control CSF from individuals who do not have this disorder or compared to predetermined standard ratios and amounts, are also provided herein.

Methods of identifying compounds that are effective for treating patients with neurodegenerative disorders characterized by deposition of cerebral amyloid by administering the compound to a subject and detecting an increase in the ratio of α -sAPP to total sAPP or an increase in the amount of α -sAPP in a sample of CSF from the subject compared to such ratio or amount in a sample of CSF prior to administering the compound are also provided herein.

Methods are provided herein for identifying and/or isolating proteases. These methods use compounds having formulae I and II in which R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , Q , n and X are as in (i)-(vii) defined above, but in which either $R_7-(Q)_n$ in formula (I) and $R_B-CH(R_A)-(Q)_n$ in formula (II) or X is replaced with a moiety, such as a chemical labeling/linking group or a chromophore or a fluorophore, useful for identifying and/or isolating proteases. For example in some embodiments, $(Q)_n$, R_7 , R_A , and R_B are as defined above and X is replaced with a chromophore or fluorophore, preferably selected from among *p*-nitranilide, 4-methoxy- β -naphthylamide, or 7-amino-4-methylcoumarin. In other embodiments, X is as defined above and the $R_7-(Q)_n$ moiety and the $(R_B)-CH(R_A)-(Q)_n$ moiety are chemical labeling/linking groups that permit detection and/or isolation of the peptide to which they are coupled. These are preferably selected from among biotin, radiolabeled moieties, fluorescein, and primary amines such as 6-amino caproic acid and amino decanoic acid.

Also provided herein are methods of identifying protease inhibitors in *in vitro* competitive inhibition assays. These methods use compounds having formulae I and II in which R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , Q and n are as in (i)-(vii) defined above, but in which X is replaced with a chromophore or fluorophore, preferably selected from among *p*-nitranilide, 4-methoxy- β -naphthylamide, or 7-amino-4-methylcoumarin. Such inhibitors may be useful in modulating the processing of amyloid precursor protein (APP).

Also provided herein are compounds having formulae I and II in which R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , Q , n and X are as in (i)-(vii) defined above, but in which either $R_7-(Q)_n$ in formula (I) and $R_8-CH(R_A)-(Q)_n$ in formula (II) or X is replaced with a moiety, such as a chemical labeling/linking group or a chromophore or a fluorophore, preferably selected from among *p*-nitranilide, 4-methoxy- β -naphthylamide, or 7-amino-4-methylcoumarin. In preferred embodiments, these compounds that include a chemical labeling/linking group or chromophore or fluorophore are as defined with the proviso that:

at least one of the amino acid residues is a non-naturally-occurring α -amino acid or at least one of R_1 , R_3 and R_5 is not a side chain of a naturally-occurring amino acid; and when X is an aldehyde, R_1 cannot be the side chain of norleucine or norvaline; or

at least one of the amino acid residues is a non-naturally-occurring α -amino acid or at least one of R_1 , R_3 and R_5 is not a side chain of a naturally-occurring amino acid; and when X is an aldehyde, the non-naturally-occurring amino acid (or side chain thereof) is other than norleucine or norvaline; or

at least one of the amino acid residues is a non-naturally-occurring α -amino acid or at least one of R_1 , R_3 and R_5 is not a side chain of a naturally-occurring amino acid; and when R_1 is the side chain from a

-13-

non-naturally-occurring amino acid, it is not the side chain of norleucine or norvaline; or

at least one of the amino acid residues is a non-naturally-occurring α -amino acid or at least one of R_1 , R_3 and R_5 is not a side chain of a naturally-occurring amino acid; and none of R_1 , R_3 and R_5 is the side chain of norleucine or norvaline.

DETAILED DESCRIPTION AND PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are incorporated by reference.

As used herein, the term "alkyl" includes the straight, branched-chain and cyclic manifestations thereof, the number of carbons atoms is generally specified. Where not specified the alkyl groups preferably contain from about 1 up to about 10 or 12, more preferably 1 to 6 or 7, and most preferably 4 to 6 carbons. Exemplary of such moieties are methyl, ethyl, propyl, cyclopropyl, isopropyl, *n*-butyl, *t*-butyl, *sec*-butyl, cyclobutyl, pentyl, cyclopentyl, *n*-hexyl, *n*-nonyl, *n*-decyl, cyclohexyl, cyclohexylmethyl, cyclohexylethyl, and the like. Lower alkyl refers to alkyl groups containing six or fewer carbon atoms.

As used herein, heteroatoms are selected from O, N or S.

As used herein, heterocycle means a ring system that includes one or more heteroatoms selected from S, O or N. Heterocycles include aliphatic rings (saturated) and heteroaryl rings. Preferred cyclic groups contain one or two fused rings and include from about 3 to about 7 members in each ring.

As used herein, the term " $NR_D R_E$ " refers to a substituent in which a nitrogen atom is bound to both the R_D and R_E substituents.

As used herein, the term " NRR' " refers to a substituent in which a nitrogen atom is bound to both the R and R' substituents.

- The term "aryl" within the definitions of X, R₈, R₁, R₃, R₆, and R₇ includes carbocyclic and heterocyclic moieties. Preferred aralkyl and aryl moieties are phenyl, benzyl, phenethyl, 1- and 2-naphthylmethyl, 1- and 2-naphthyl, 2-, 3-, 4- pyridyl, 2- and 3-furyl, 1- and 2-indenyl, 1- and 2-thiophenyl, imidazolyl, indolyl, 2- and 3-thienyl, indole-3-ethyl and the residue of 1,2,3,4-tetrahydroisoquinoline. Other carbocycles are such fused moieties as pentalenyl, indenyl, naphthalenyl, naphthylmethyl, azulenyl, heptalenyl, acenaphthylenyl, 9-fluorenyl, phenalenyl, phenanthrenyl, anthracenyl, triphenylenyl, pyrenyl, chrysenyl, and naphthacenyl.
- 10 Exemplary of alkynyl is propynyl. Exemplary of alkenyl moieties are 2-methyl-2-propenyl, 2-methyl-1-propenyl, propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 2,2-difluoroethenyl, as well as those straight and branched chained moieties having up to two double bonds. Cyclic carbon moieties preferably contain one or two fused rings typically from 3 up to about 16, preferably
- 15 4 up to about 12 carbons.

- Haloalkyl embraces such moieties as CF₃, -CF₂H, -CFH₂, CH₂Cl and CH₂Br and other halo substituted lower alkyls. Exemplary of aryloxyalkenyl and aryloxyalkynyl moieties of R_A are phenoxymethyl, CF₃-substituted phenoxymethyl, benzyloxymethyl, phenoxybutyr-2-ene, 1-phenyl-1-propene,
- 20 CF₃-phenoxybutyr-2-ene, CF₃-benzyloxymethyl, these moieties are preferred when R_A is other than R₁.

- In those instances in which a substituent, such as the R₁, R₃, and/or R₅ moiety, embrace the residue -or side chain- of a naturally occurring α -amino acid, it is to be noted that each α -amino acid has a characteristic "R-group," the R-group being the residue -or side chain- attached to the α -carbon atom of the amino acid. For example, the residue of glycine is H, for alanine it is methyl, for valine it is isopropyl. The specific residues of the naturally occurring α -amino acids are well known to those of skill in this art [see, e.g., A. L. Lehninger, *Biochemistry: The Molecular Basis of Cell*
- 25

-15-

Structure and Function, 1970 (or any edition thereafter), Worth Publishers, NY, see, particularly Chapter 4).

As used herein, the residues of naturally occurring α -amino acids are the residues of those 20 α -amino acids found in nature which are incorporated into protein by the specific recognition of the charged tRNA molecule with its cognate mRNA codon in humans.

As used herein, non-naturally occurring amino acids refer to amino acids that are not genetically encoded. Preferred such non-naturally occurring amino acids herein include those with unsaturated side chains.

As used herein, certain moieties in the peptides and peptide analogs provided herein are designated according to the system of nomenclature in which the binding region of a proteinase is considered as a series of subsites, S, along the surface of the enzyme [see, Schechter and Berger (*Biochem. Biophys. Res. Comm.*, 27, 157-162 (1967))]. Each subsite binds an individual peptide residue, P. This system of nomenclature, which was originally designed for papain, has been adapted to other proteases. Thus, for convenience and in keeping with the customary peptide designations, the moiety bearing the R_1 side chain (or residue) is designated as the P_1 moiety, the moiety bearing the R_2 side chain (or residue) is designated as the P_2 moiety, and that which bears the R_3 moiety is designated as the P_3 moiety.

When the $R_7-(Q)_n$ - and $(R_8)-CH(R_A)-(Q)_n$ - moieties are as defined in any of (i)-(vii) above, they are referred to as N-terminal capping, or blocking, moieties and include those moieties that protect molecules from degradation by aminopeptidases including, but not limited to, such generic groupings as arylcarbonyl, alkylcarbonyl, alkoxycarbonyl, aryloxy carbonyl, aralkyloxy carbonyl, aralkylsulfonyl, alkylsulfonyl, arylsulfonyl, and other equivalently functioning groups known in the art.

As defined particularly for the capping groups herein, either individually or as a part of a larger group, "alkyl" means a linear, cyclic, or branched-chain aliphatic moiety of 1 to 20 carbon atoms; "aryl" means an

aromatic moiety, e.g., phenyl, of 6 to 18 carbon atoms, unsubstituted or substituted with one or more alkyl, substituted alkyl, nitro, alkoxy, or halo groups; "substituted alkyl" means an alkyl group having a substituent containing a heteroatom or heteroatoms such as N, O, or S; "halo" means
5 Cl or Br; and "alkaryl" means an aryl moiety of 6 to 19 carbon atoms having an aliphatic substituent, and, optionally, other substituents such as one or more alkyl, substituted alkyl, alkoxy or amino groups.

Examples of suitable N-terminal blocking groups include, but are not limited to, formyl, *t*-butoxycarbonyl, isopropoxycarbonyl, allyloxycar
10 bonyl, acetyl, trifluoroacetyl, methyl, ethyl, benzyl, benzoyl, acetoacetyl, chloroacetyl, succinyl, phthaloxy, phenoxycarbonyl, methoxysuccinyl, *p*-methoxybenzenesulfonyl, *p*-toluenesulfonyl, isovaleroyl, methanesulfonyl, benzyloxycarbonyl, substituted benzyloxycarbonyl, adipyl, suberyl, phthal-
amido-, morpholino-, azeloyl, dansyl, tosyl, 2,4-dinitrophenyl, fluorenyl-
15 methoxycarbonyl, methoxyazeloyl, methoxyadipyl, methoxysuberyl, 1-adamantanesulfonyl, 1-adamantaneacetyl, 2-carbobenzoyl, phenylacetyl, *t*-butylacetyl, bis[(1-methyl)methyl]acetyl, and thioproline.

As used herein, moieties useful in methods of identifying and/or isolating proteases include, but are not limited to, chemical labeling/linking
20 groups, chromophores and fluorophores.

As used herein, a chromophore is a chemical group that absorbs light at a specific frequency when in a free state (i.e., uncoupled from the peptide compounds described herein) and thereby imparts a detectable color (e.g., detectable spectrophotometrically). Chromophores suitable for use in
25 generating chromogenic substrates are well known to those of skill in the art. Preferred chromophores include *p*-nitroanilide, 4-methoxy- β -naphthylamide and para- or ortho-nitrophenols.

As used herein, a fluorophore is a chemical group that, when in a free state (i.e., uncoupled from the peptide compounds described herein),
30 absorbs radiation of one wavelength and emits radiation of a different

-17-

wavelength which is detectable using standard fluorometric techniques. The process of fluorescence refers to emission of a photon by a molecule in an excited singlet state. Fluorophores suitable for use in generating fluorogenic substrates are well known to those of skill in the art [see, for example, 5 Nicholson *et al.* Nature 376:37-43 (1995)] and include 7-amino-4-methylcoumarin and 5(6)-carboxy-fluorescein with 7-amino-4-methylcoumarin being preferred.

As used herein, a chromogenic or fluorogenic substrate is a molecule containing a chromophore or fluorophore that does not emit color or 10 fluorescence. Alteration of the substrate in such a way as to effect removal of the chromophore or fluorophore from the remainder of the substrate molecule results in emission of color or fluorescence from the released free chromophore or fluorophore. Chromogenic and fluorogenic substrates are represented by any of the compounds of formulae I and II in which X is a 15 chromophore or fluorophore.

As used herein, chemical labeling/linking groups are chemical moieties characterized by being readily detectable through a variety of means, e.g., spectrophotometry, fluorometry, radiography, scintillation counting. The labeling group includes a label portion which is bonded to a chemical linker 20 which, in turn, is bonded to the molecule being labeled. The labeling group can be detectable in and of itself or through formation of a complex with a detectable moiety. Chemical labeling groups are well known to those of skill in the art and include derivatives of biotin (which may be detected by binding to streptavidin conjugated to horseradish peroxidase). Chemical 25 linking groups are chemical moieties that permit efficient coupling of the molecule to which they are linked to solid supports. The chemical linking groups include chemical linkers contained in chemical labeling groups as described above and are preferably primary amines such as 6-amino caproic acid and amino decanoic acid.

As used herein, an effective amount of a compound for treating a disorder is an amount that is sufficient to ameliorate, or in some manner reduce a symptom or stop or reverse progression of a condition. Such amount may be administered as a single dosage or may be administered according to a regimen, whereby it is effective.

As used herein, treatment means any manner in which the symptoms or pathology of a condition, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein.

As used herein, amelioration of the symptoms of a particular disorder by administration of a particular pharmaceutical composition refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography [TLC], gel electrophoresis and high performance liquid chromatography [HPLC], used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

As used herein, biological activity refers to the in vivo activities of a compound or physiological responses that result upon in vivo administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such

-19-

compounds, compositions and mixtures. Biological activity may be detected by in vitro assays, such as those described herein.

As used herein, pharmaceutical activity refers to the activity of the compounds herein to treat a disorder.

- 5 As used herein, the IC_{50} refers to an amount, concentration or dosage of a particular compound that achieves a 50% inhibition of a maximal response.

- 10 As used herein, EC_{50} refers to a dosage, concentration or amount of a particular test compound that elicits a dose-dependent response at 50% of maximal expression of a particular response that is induced, provoked or potentiated by the particular test compound.

- 15 As used herein, a prodrug is a compound that, upon *in vivo* administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, the pharmaceutically active compound is modified such that the active compound will be regenerated by metabolic processes. The prodrug may be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism *in vivo*, once a pharmaceutically active compound is identified, those of skill in the pharmaceutical art generally can design prodrugs of the compound [see, e.g., Nogrady (1985) Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-392].
- 20

- 25 As used herein, amyloid precursor protein (APP) is the progenitor of deposited amyloidogenic $A\beta$ peptides ($A\beta$) found in the senile plaques in patients with diseases, such as Alzheimer's disease (AD), that are characterized by such deposition. α -sAPP is an alternative cleavage product of APP; its formation precludes formation of $A\beta$. Total sAPP (often

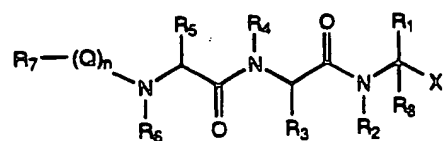
designated as sAPP) refers to any soluble N-terminal fragment of APP, including α -sAPP, that is released upon cleavage of APP.

As used herein, Cha is cyclohexylalanine, and Chg is cyclohexylglycine.

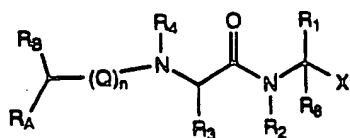
5 As used herein, the abbreviations for any substituent groups, protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature [see, (1972) Biochem. 11:1726].

10 A. The tri- and dipeptide analogs

Compounds of formulae (I) and (II):



I



II

or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof are provided, but with the proviso that, when the compounds have formula (I): (1) at least one of the amino acid residues in the resulting tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R_1 , R_3 and R_5 is not a side chain of a

naturally-occurring amino acid; and (2) when X is an aldehyde, the non-naturally occurring amino acid (or side chain thereof) is other than norleucine or norvaline, and when the compounds have formula (II) and X is an aldehyde, R₁ cannot be the side chain of norleucine or norvaline.

- 5 In some embodiments the compounds of formula (II) are also selected such that: (1) at least one of the amino acid residues in the resulting dipeptide is a non-naturally-occurring α -amino acid or at least one of the R₁ and R₃ is not a side chain of a naturally-occurring amino acid; and (2) when X is an aldehyde, the non-naturally occurring amino acid (or side chain thereof) is other than norleucine or norvaline.
- 10

- In certain preferred embodiments, the compounds have formulae I or II, particularly formula I, as defined above, but with the proviso that: (1) at least one of the amino acid residues in the resulting di or tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R₁, R₃ and R₅ is not a side chain of a naturally-occurring amino acid; and (2) when R₁ is the side chain from a non-naturally occurring amino acid, it is not the side chain of norleucine or norvaline.
- 15

- In other preferred embodiments, the compounds have formulae I or II, particularly formula I, as defined above, but with the proviso that: (1) at least one of the amino acid residues in the resulting di or tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R₁, R₃ and R₅ is not a side chain of a naturally-occurring amino acid; and (2) none of R₁, R₃ and R₅ is the side chain of norleucine or norvaline.
- 20

- R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, X, Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi), (vii) as described above.
- 25

Preferred among these compounds, defined with any of the provisos, are those in which:

- R₁ is preferably a straight or branched chain carbon chain containing 2 to 6 carbons and one unsaturated, preferably a double, bond or is a cyclic moiety containing from 5 to 6 members, and is more preferably 2-methyl
- 30

propene, 2-butene, cyclohexyl, lower alkyl-substituted cyclohexyl or cyclohexylmethyl;

R_2 , R_4 , and R_6 are each independently selected from among H or C_{1-4} alkyl, and more preferably methyl or ethyl;

- 5 R_3 is C_{1-4} alkyl, phenyl, naphthyl, hydroxyphenyl, 1-aminobutyl, acetamide, and more preferably *iso*-butyl, benzyl, or phenyl;

R_5 is C_{1-4} alkyl, and more preferably *iso*-butyl;

R_6 is H or C_{1-4} alkyl, and more preferably H or methyl;

- 10 $R_7 - (Q)_n$ is selected from acyl (Ac), benzyloxycarbonyl (Cbz), 9-fluor-enylmethylcarbonate (Fmoc), Boc, tosyl, with Cbz, Ac and Fmoc being more preferred, and Cbz and Ac most preferred;

Q is $-C(O)-$, $-S(O)_2-$ and $-O-C(O)-$, with $-C(O)-$ and $-O-C(O)-$ being more preferred, and $-O-C(O)-$ most preferred;

R_8 is C_{1-4} alkyl or C_{2-4} alkenyl, and more preferably *iso*-butyl;

- 15 $R_A = -(T)_m-(D)_n-R_1$, in which T is oxygen or nitrogen, with oxygen being more preferred, and D is C_{1-4} alkyl or C_{2-4} alkenyl, with a mono-unsaturated C_{3-4} alkenyl being more preferred; and

X is aldehyde, α -ketoester, α -ketoamide, trifluoromethylketone, diazomethylketone, or nitrile, with an aldehyde, α -ketoester or α -ketoamide

- 20 being more preferred.

Also among preferred compounds are those of formula (II) in which R_B , R_A and the atom to which each is attached and $(Q)_n$ form (2*SR*)-*N*-[(2*S*)-2-benzyloxy-4-methylpentanoyl] or (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl] or valeroyl.

- 25 Also among preferred compounds are those of formula (I) in which R_5 and R_6 and the atoms to which each is attached form a 4-6 membered heterocyclic moiety that is preferably pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl.

-23-

When R_1 , R_3 , and/or R_6 are a side-chain from other than a residue of a naturally occurring α -amino acid, it is preferred that such moiety is a straight or branched carbon chain, preferably containing at least one unsaturated bond, preferably a double bond, and 2 to 10, preferably 4 to 7, more preferably 4-6 carbon atoms in the chain, such as, but not limited to, 2-methyl propene and 2-butene, or is a cyclic moiety, preferably containing 4-6 members, more preferably is cyclohexylmethyl. The resulting residues including such moieties include, but are not limited to, 2-amino-4-methyl-4-pentenoic acid, 2-amino-4-hexenoic acid, cyclohexylalanine and cyclohexylglycine; (2S)-2-amino-4-methyl-4-pentenoic acid and (2S)-2-amino-4-hexenoic acid are preferred.

When the compounds are used in the methods of treating neurodegenerative diseases and cognitive disorders provided herein, the side chains from norvaline and norleucine are also preferred.

In particular, preferred compounds are those in which at least one of R_1 , R_3 , and R_6 is 2-methyl-propene, 2-butene, cyclohexyl or cyclohexylmethyl. More preferred are those in which R_1 , R_3 , and R_6 are independently selected from 2-methyl-propene, 2-butene, cyclohexyl or cyclohexylmethyl, and X is $C(O)H$, $C(O)-C(O)OR_D$, $C(O)-C(O)-NR_D R_E$, $C(O)CH_2CHN_2$, $C(O)CF_3$, or $C\equiv N$.

Preferred heterocyclic ring moieties containing R_1 and R_2 and the atoms to which they are attached, when R_6 is H, are morpholino, thiomorpholino, pyrrolidiny, or V-substituted pyrrolidiny, particularly 4-hydroxy pyrrolidiny.

Preferred heterocyclic ring moieties containing R_6 and R_7 and the atoms to which they are attached when $(Q)_n$ is a carbonyl group are selected from among succinimide, phthalimide or maleimide, with phthalimide being more preferred.

Preferred heterocyclic ring moieties containing R_6 and R_7 and the atoms to which they are attached when n in $(Q)_n$ is zero are morpholino, thiomorpholino, pyrrolidinyl, V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl, or 1,2,3,4-tetrahydroisoquinoline.

- 5 Preferred moieties, when n is zero, and when R_3 and R_4 or R_5 and R_7 are taken together with the atoms to which they are attached form heterocyclic moieties are morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl.

- 10 Another preferred moiety when R_1 and R_2 , R_3 and R_4 , R_5 and R_7 , and/or R_6 and R_7 together with the atoms to which each is attached form a heterocyclic moiety is 4-hydroxy pyrrolidinyl.

- The following are among the preferred compounds provided herein:
- N*-Ac-L-Leu-L-Leu-DL-cyclohexylalaninal; (2*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[2-(4-methyl-4-pentenal)]amide; (2*SR*)-H-L-Leu *N*-[2-(ethyl 4-methyl-4-penteno-
- 15 ate)]amide hydrochloride; (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl]-L-Leu *N*-[2-(4-methyl-4-pentenal)]amide; (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu-*N*-[2-(4-methyl-4-pentenal)]amide; *N*-Cbz-L-Leu-L-Leu-DL-cyclohexylglycinal; *N*-Ac-L-Leu-L-Leu-DL-cyclohexylglycinal; (2*SR*)-*N*-Ac-L-Leu-L-Leu *N*-[2-(*trans*-4-hexenal)]amide; (2*SR*)-*N*-Ac-L-Leu-L-Leu *N*-[2-
- 20 (4-methyl-4-pentenal)]amide; (2*SR*)-*N*-Cbz-L-Leu-L-Phe-DL-cyclohexylalaninal; and (2*SR*)-*N*-Cbz-L-Leu-L-Phe-*N*-[2-(4-methyl-4-pentenal)]amide; *N*-Ac-L-Leu-L-Leu-L-Nle-COCHN₂; (2*S*)-*N*-Ac-L-Leu-L-Leu *N*-[2-(pentanenitrile)]amide; (2*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[2-(4-methyl-4-pentenal)]amide; (2*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu-*N*-[2-(4-methyl-4-pentenal)]amide; (2*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[2-(*trans*-4-hexenal)]amide; (2*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu-*N*-[2-
- 25 (*trans*-4-hexenal)]amide.

- Also provided herein are the α -keto esters and α -keto amides of any of the above-listed compounds. For example, such compounds include
- (3*SR*)-*N*-Cbz-L-Leu-L-Leu-*N*-[3-(methyl 2-keto-5-methyl-3-hexenoate)]amide;
- 30 (3*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenoate)] amide;

- (3*SR*)-*N*-Cbz-L-Leu-L-Leu*N*-[3-(benzyl 2-keto-5-methyl-3-hexenoate)] amide;
 (3*SR*)-*N*-Cbz-L-Pro-L-Leu*N*-[3-(methyl 2-keto-5-methyl-3-hexenoate)] amide;
 (3*SR*)-*N*-Cbz-L-Pro-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenoate)] amide;
 (3*SR*)-*N*-Cbz-L-Pro-L-Leu*N*-[3-(benzyl 2-keto-5-methyl-3-hexenoate)] amide;
 5 (3*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenamide)]
 amide; (3*SR*)-*N*-Cbz-L-Leu-L-Leu*N*-[3-(ethyl 2-keto-5-methyl-3-hexenamide)]
 amide; (3*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-
 hexenamide)] amide; (3*SR*)-*N*-Cbz-L-Pro-L-Leu*N*-[3-(methyl 2-keto-5-methyl-
 3-hexenamide)] amide; (3*SR*)-*N*-Cbz-L-Pro-L-Leu*N*-[3-(ethyl 2-keto-5-methyl-
 10 3-hexenamide)] amide, (3*SR*)-*N*-Cbz-L-Pro-L-Leu *N*-[3-(benzyl 2-keto-5-
 methyl-3-hexenamide)] amide; (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-(methyl
 2-keto-5-methyl-3-hexenoate)] amide; (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-
 (ethyl 2-keto-5-methyl-3-hexenoate)] amide; (3*SR*)-*N*-Cbz-L-HydroxyPro-L-
 Leu *N*-[3-(benzyl 2-keto-5-methyl-3-hexenoate)] amide; (3*SR*)-*N*-Cbz-L-
 15 HydroxyPro-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenamide)] amide;
 (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenamide)]
 amide; (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-
 hexenamide)] amide; (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-
 methylpentanoyl]]-L-Leu*N*-[3-(methyl 2-keto-5-methyl-3-hexenoate)] amide;
 20 (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[3-
 (ethyl 2-keto-5-methyl-3-hexenoate)] amide; (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-
 propene)-4-methylpentanoyl]]-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-
 hexenoate)] amide; (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-
 methylpentanoyl]]-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenamide)]
 25 amide; (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu*N*-
 [3-(ethyl 2-keto-5-methyl-3-hexenamide)] amide; (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-
 1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-
 hexenamide)] amide; (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl]-L-Leu*N*-
 [3-(methyl 2-keto-5-methyl-3-hexenoate)] amide; (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-
 30 methylpentanoyl]-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenoate)] amide;

(2SR)-N-[(2S)-2-benzoxo-4-methylpentanoyl]-L-Leu N-[3-(benzyl 2-keto-5-methyl-3-hexenoate)] amide; (2SR)-N-[(2S)-2-benzoxo-4-methylpentanoyl]-L-Leu N-[3-(methyl 2-keto-5-methyl-3-hexenamide)] amide; (2SR)-N-[(2S)-2-benzoxo-4-methylpentanoyl]-L-Leu N-[3-(ethyl 2-keto-5-methyl-3-hexenamide)] amide; (2SR)-N-[(2S)-2-benzoxo-4-methylpentanoyl]-L-Leu N-[3-(benzyl 2-keto-5-methyl-3-hexenamide)] amide.

Also provided herein are any of the above-listed compounds in which R₂, and/or R₄ and/or R₆ are methyl (i.e., the N-methyl derivatives of the preferred compounds).

10 The following, in addition to all the above-listed compounds, are among the preferred compounds for use in the methods herein: N-Cbz-L-Leu-L-Leu-DL-(methyl)norleucinal; N-Cbz-L-Leu-L-Leu-DL-Nle-CO₂Et; N-Cbz-L-Leu-L-Leu-DL-Nle-CO₂H; N-Cbz-L-Leu-L-Leu-DL-Nle-CONHEt; (2S)-N-Cbz-L-Leu-L-Leu N-[2-(thiazole)-oxo-pentyl]amide; N-Ac-L-Leu-L-Leu-L-Nle-COCHN₂;
 15 (3SR)-N-Ac-L-Leu-L-Leu N-[3-(1,1,1-trifluoro-2-oxo-heptyl)]amide; (2SR)-H-L-Leu N-[2-(ethyl 4-methyl-4-pentenoate)]amide hydrochloride; (2SR)-(3S)-N-Cbz-L-Leu-L-Leu N-[3-(2-hydroxy-heptanoic acid)]amide; (2SR)-(3S)-N-Cbz-L-Leu-L-Leu N-[3-(methyl 2-hydroxy-heptanoate)]amide; (2SR)-(3S)-N-Cbz-L-Leu-L-Leu N-[3-(benzyl 2-hydroxy-heptamide)]amide; (3SR)-(4S)-N-Cbz-L-Leu-L-Leu N-[4-(benzyl 3-hydroxy-octamide)]amide; (3S)-N-Cbz-L-Leu-L-Leu N-[3-(1-furfylthio-2-oxo-heptane)]amide; N-Cbz-L-Pro-L-Leu-L-norleucinal; N-Fmoc-Leu-L-Leu-DL-norleucinal; (2SR)-N-Ac-L-Leu-L-Leu N [2-(trans-4-hexenal)]amide; N-Ac-L-Leu-L-Phe-DL-norleucinal; N-Cbz-L-Leu-L-Leu-L-norleucinal; (2SR)-N-Ac-L-Leu-L-Leu N-[2-(4-methyl-4-pentenal)]amide; N-Cbz-L-Leu-L-(methyl)Leu-DL-norleucinal; N-Dansyl-L-Leu-L-Leu-DL-norleucinal;
 20 N-Ac-L-Phe-L-Leu-DL-norleucinal; (2SR)-N-Cbz-L-Leu-L-Phe-N-[2-(4-methyl-4-pentenal)]amide; (2S)-N-Ac-L-Leu-L-Leu-N-[2-(4-methyl-pentenitrile)]amide; (2S)-N-Ac-L-Leu-L-Leu-N-[2-(pentenenitrile)]amide; (2SR)-N-Cbz-L-Pro-L-Leu-N-[2-(4-methyl-4-pentenitrile)]amide.

-27-

Also included among the compounds intended for use in the methods herein are the following: *N*-Ac-L-Leu-L-Leu-phenylalaninal; *N*-Cbz-L-Leu-L-Leu-L-norleucinal; (2*S*)-*N*-Ac-L-Leu-L-Leu-prolinal; (2*S*)-*N*-Ac-L-Leu-L-Leu-L-leucinal.

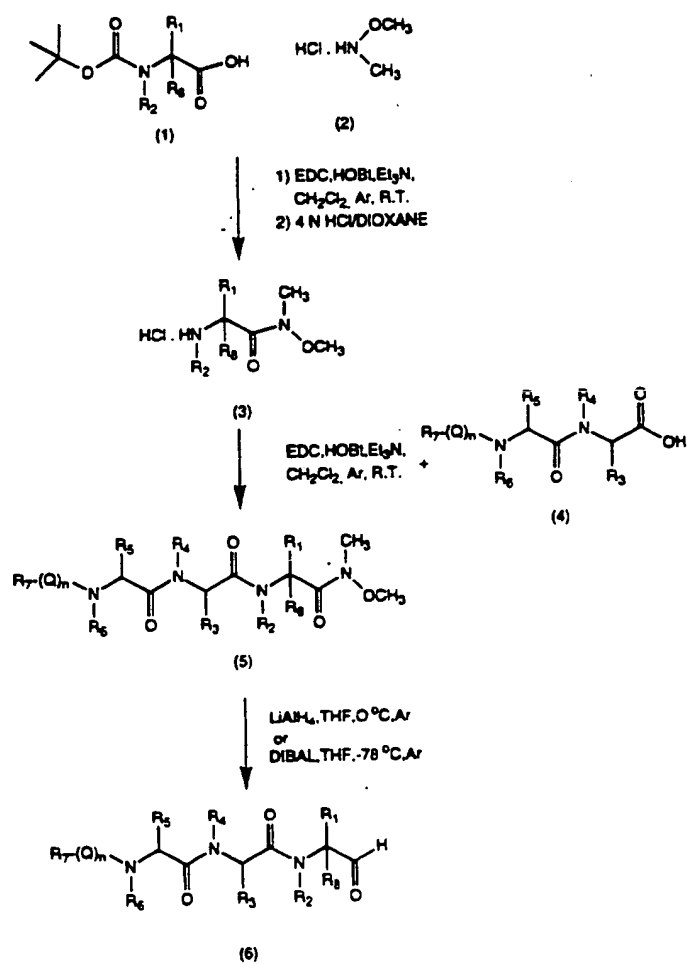
- 5 Also intended for use herein are the α -keto esters and α -keto amides of any of the above-listed compounds. Further intended for use herein are any of the above-listed compounds in which R_2 and/or R_4 and/or R_6 are methyl (i.e., the *N*-methyl derivatives of the compounds).

B. Synthesis of the tri- and dipeptide analogs

10 **1. Reaction schemes**

The following reaction schemes are depicted to illustrate the construction of the peptides provided herein and to illustrate the variety of reactions that may be used to prepare the intermediates from which compounds of formulae I and II may be prepared.

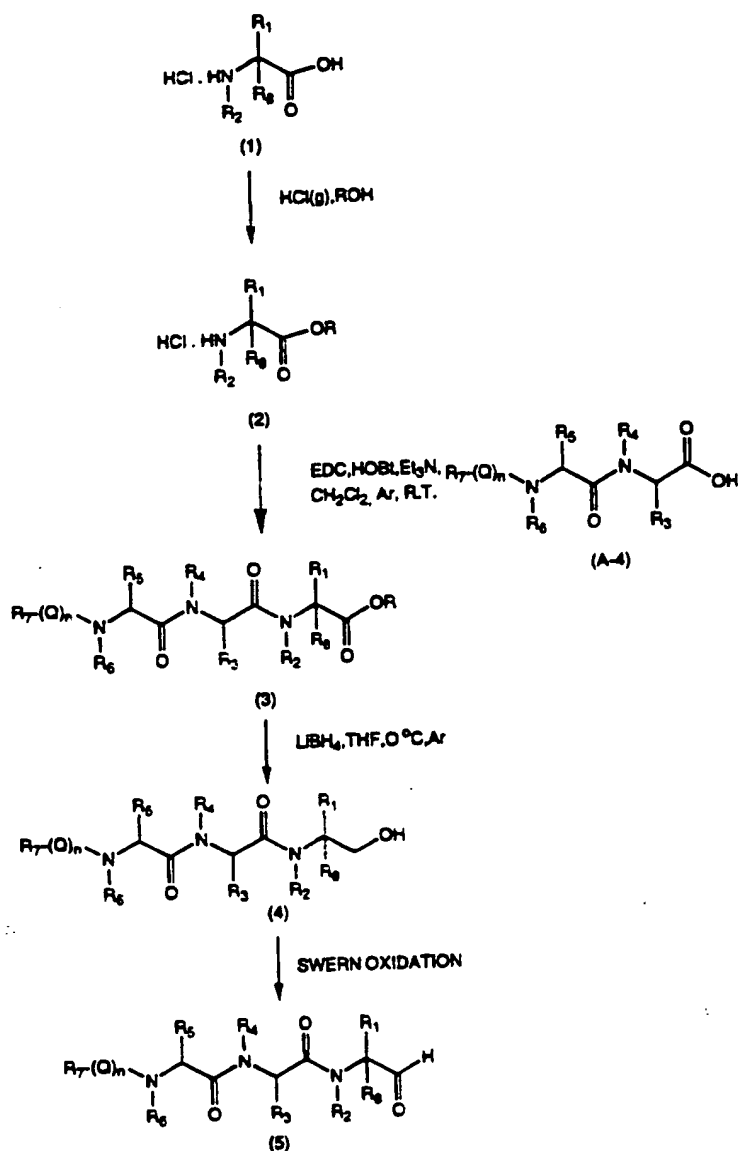
REACTION SCHEME A



wherein the R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and (O)_n are as defined above.

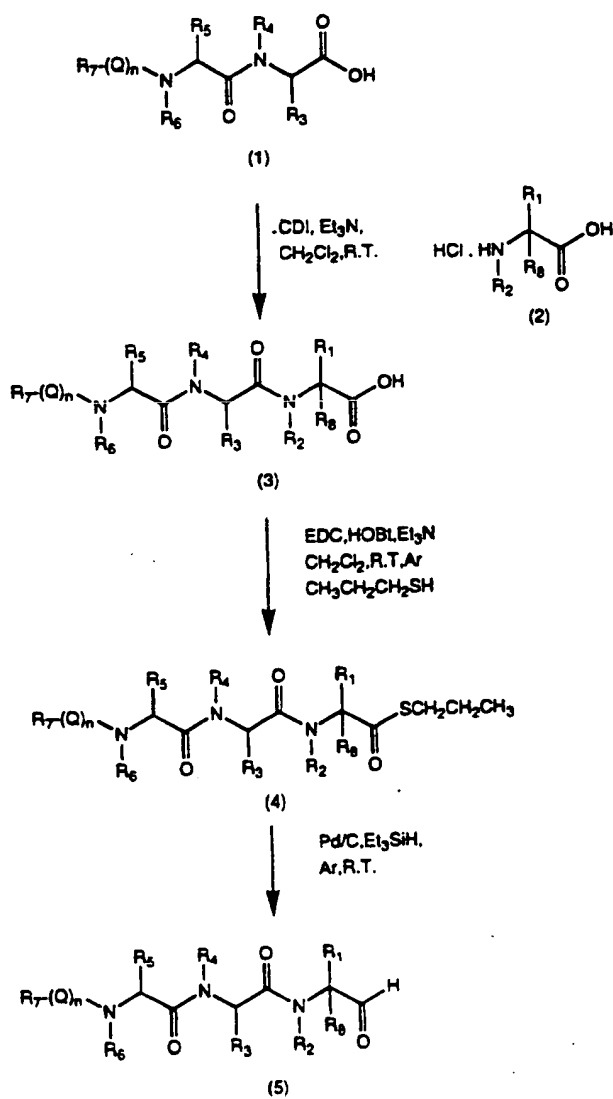
-29-

REACTION SCHEME B



wherein the $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5, \text{R}_6, \text{R}_7, \text{R}_8$ and $(\text{Q})_n$ are as defined above.

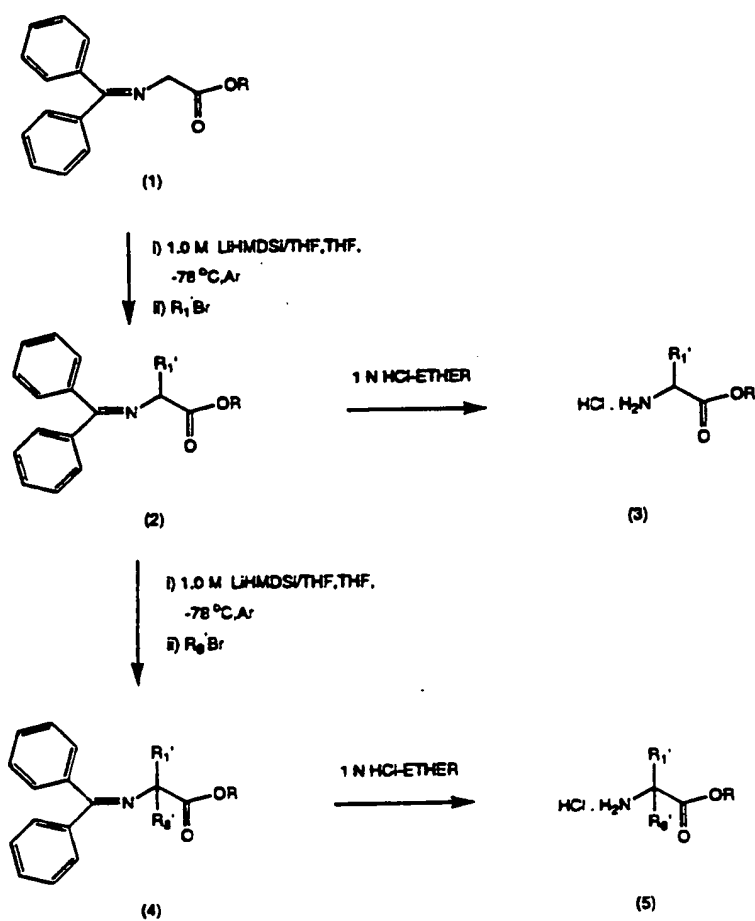
REACTION SCHEME C



wherein the $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5, \text{R}_6, \text{R}_7, \text{R}_8$ and $(\text{Q})_n$ are as defined above.

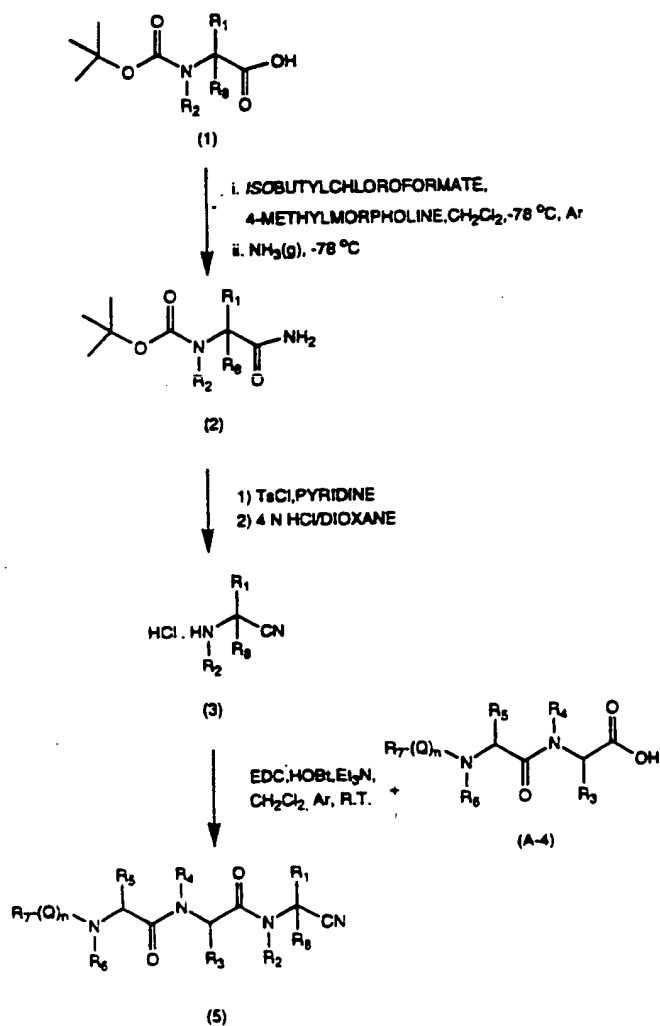
-31-

REACTION SCHEME D



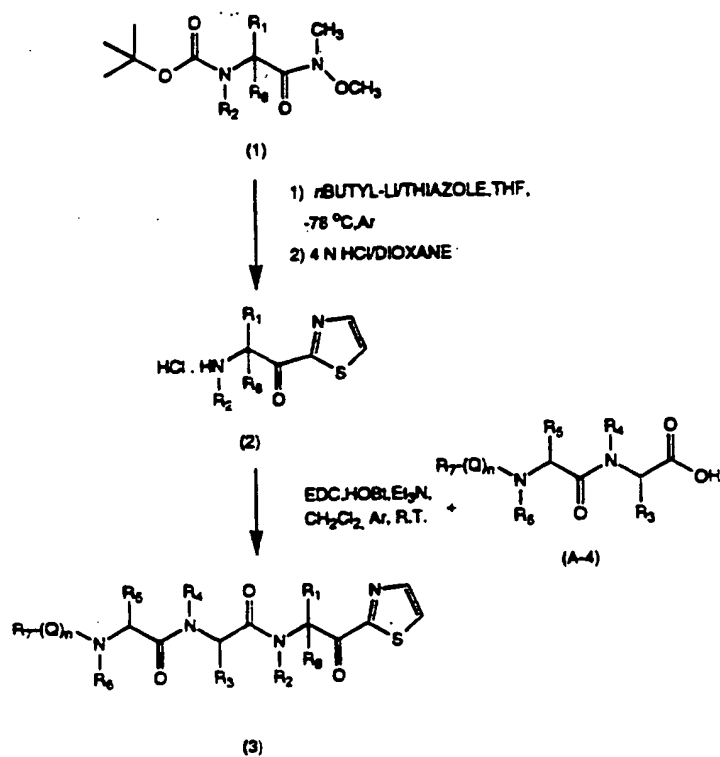
wherein R_1' is a moiety of R_1 which is not a side chain of natural α -amino acid.
 R_6' is C_{1-4} alkyl. R is methyl or ethyl.

REACTION SCHEME E



wherein the R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and (Q)_m are as defined above.

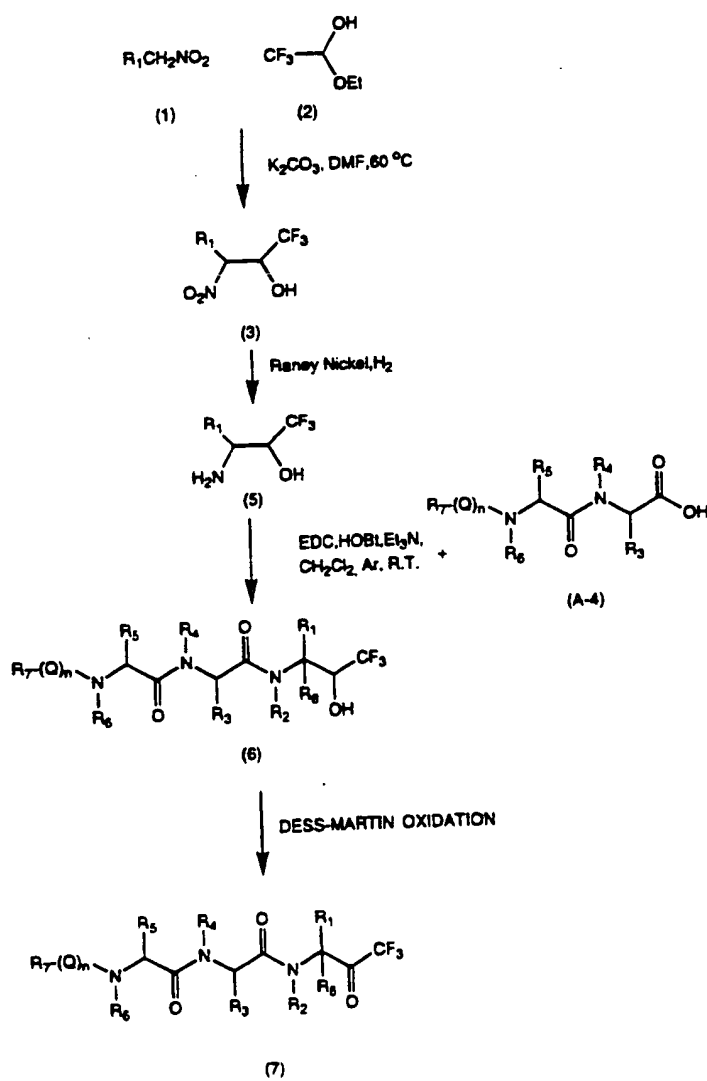
REACTION SCHEME F



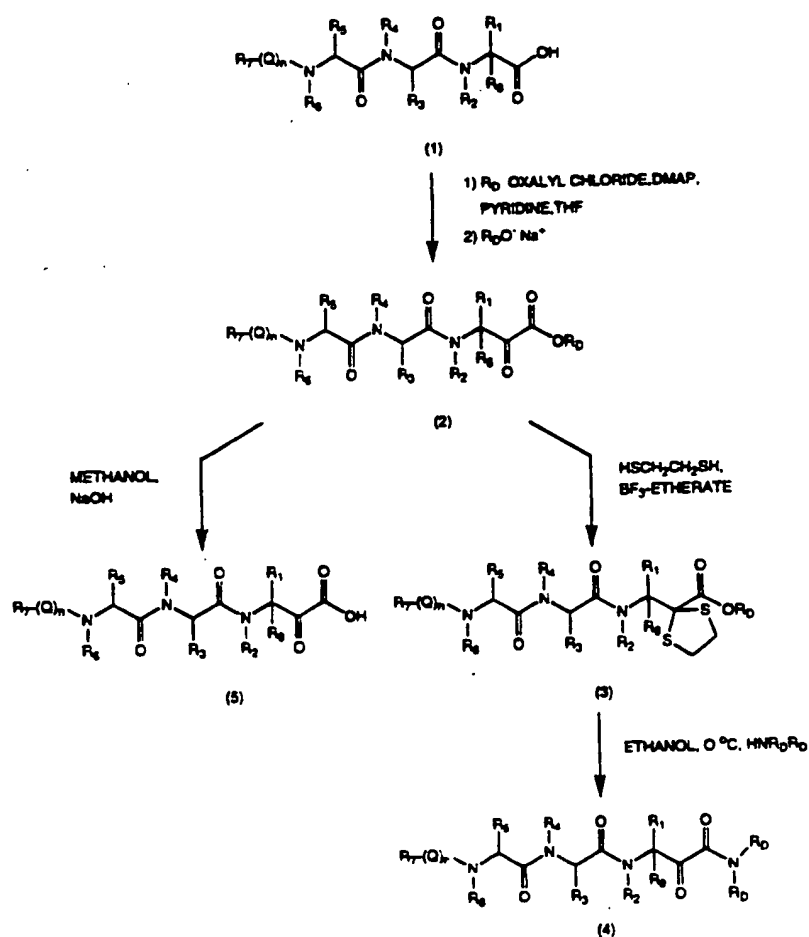
wherein the R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and (Q)_n are as defined above.

-34-

REACTION SCHEME G



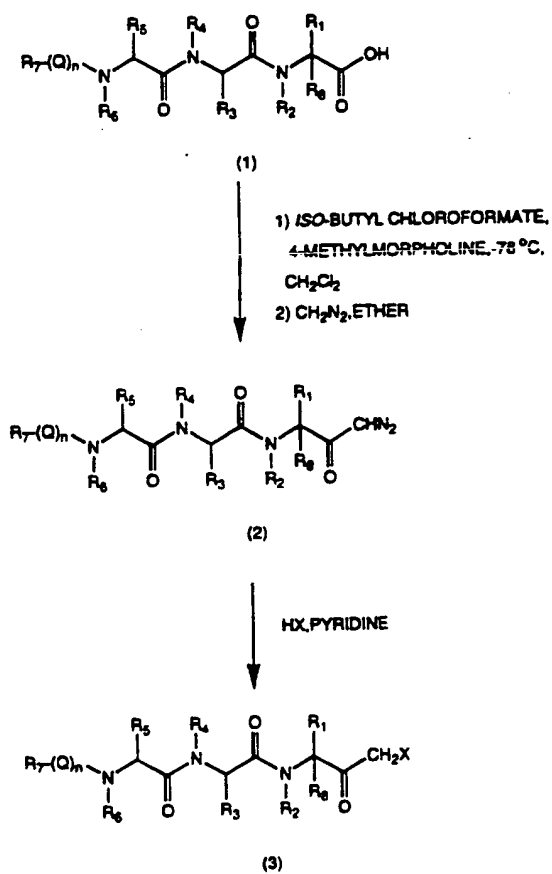
REACTION SCHEME H



wherein the $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8$ and $(Q)_m$ are as defined above.
 wherein R_0 is hydrogen, methyl, ethyl or benzyl.

-36-

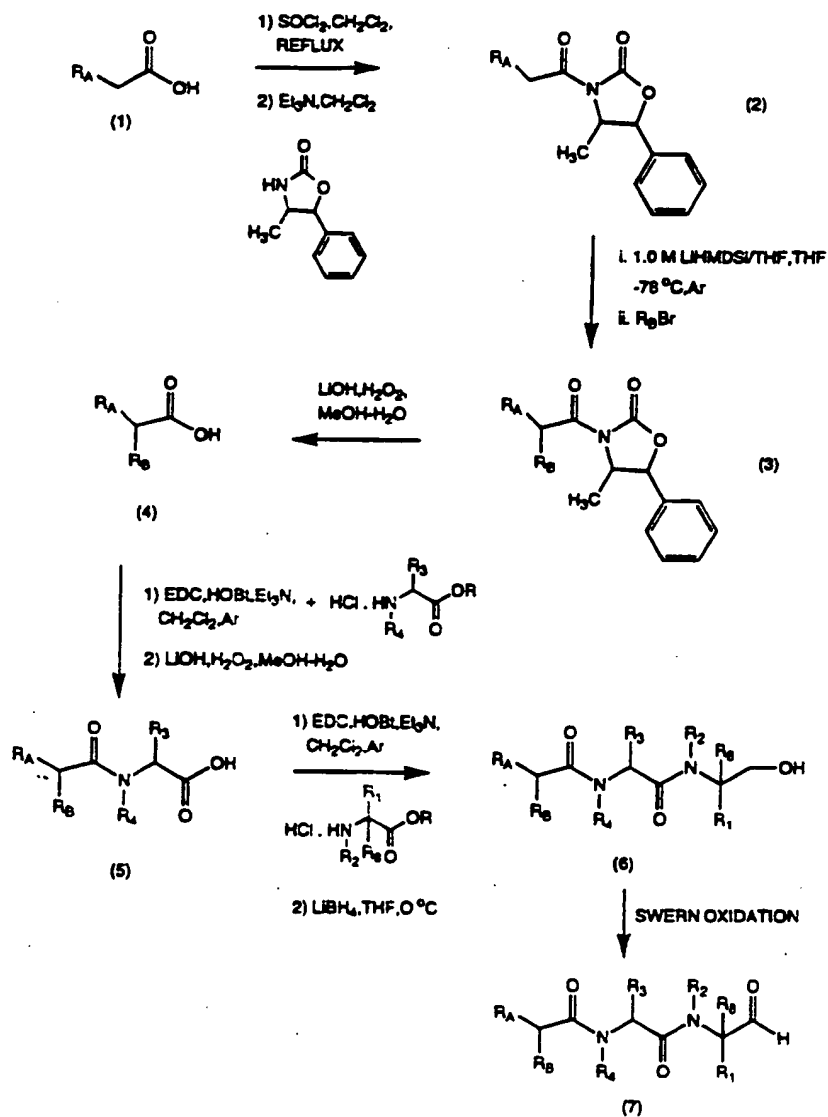
REACTION SCHEME I



wherein the R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and (Q)_n are as defined above.
wherein X is F, Cl or Br.

-37-

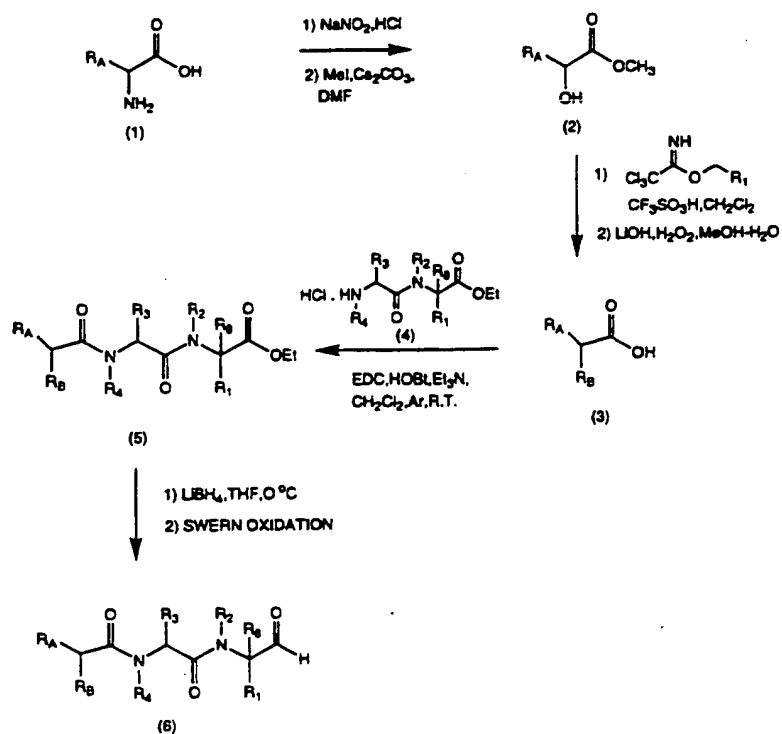
REACTION SCHEME J



wherein the $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5, \text{R}_6, \text{R}_7, \text{R}_8$ and R_9 are as defined above.

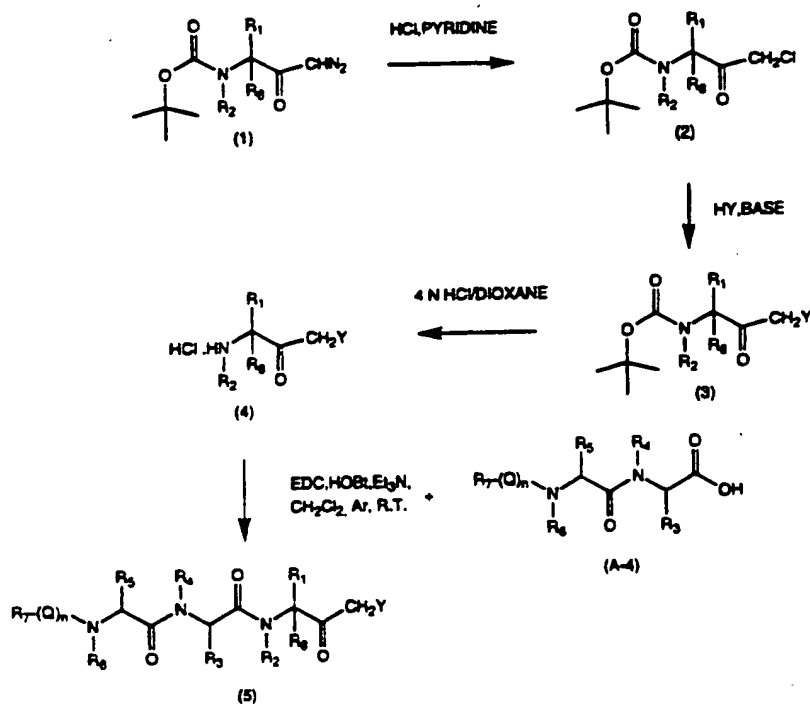
-38-

REACTION SCHEME K

wherein the $R_1, R_2, R_3, R_4, R_5, R_A$ and R_B are as defined above.

-39-

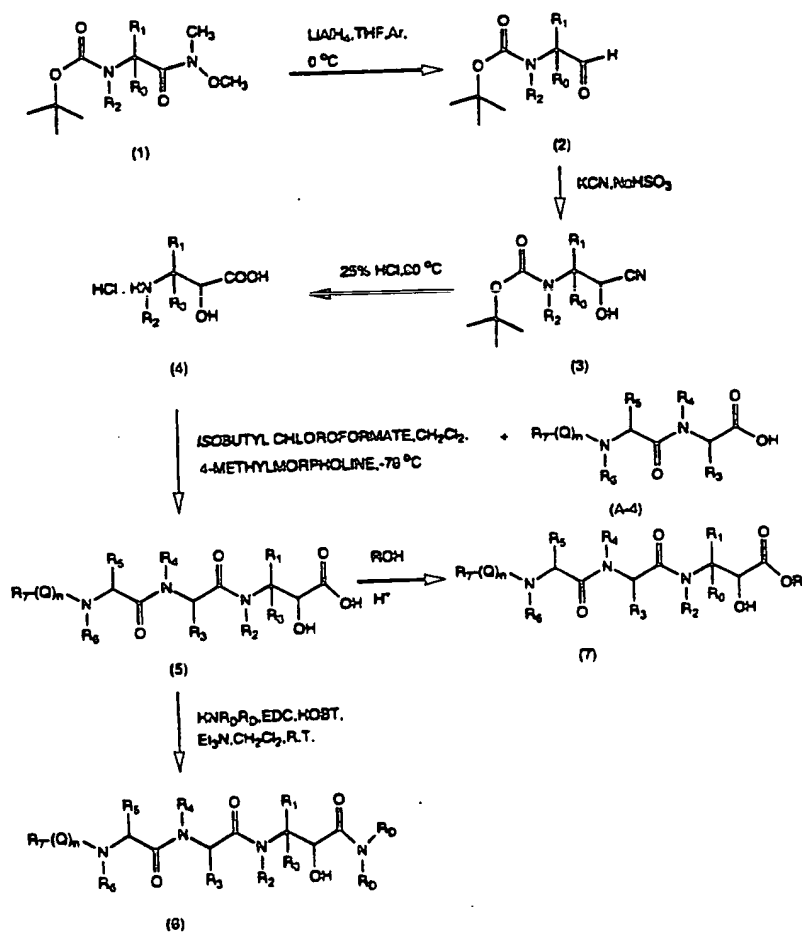
REACTION SCHEME L



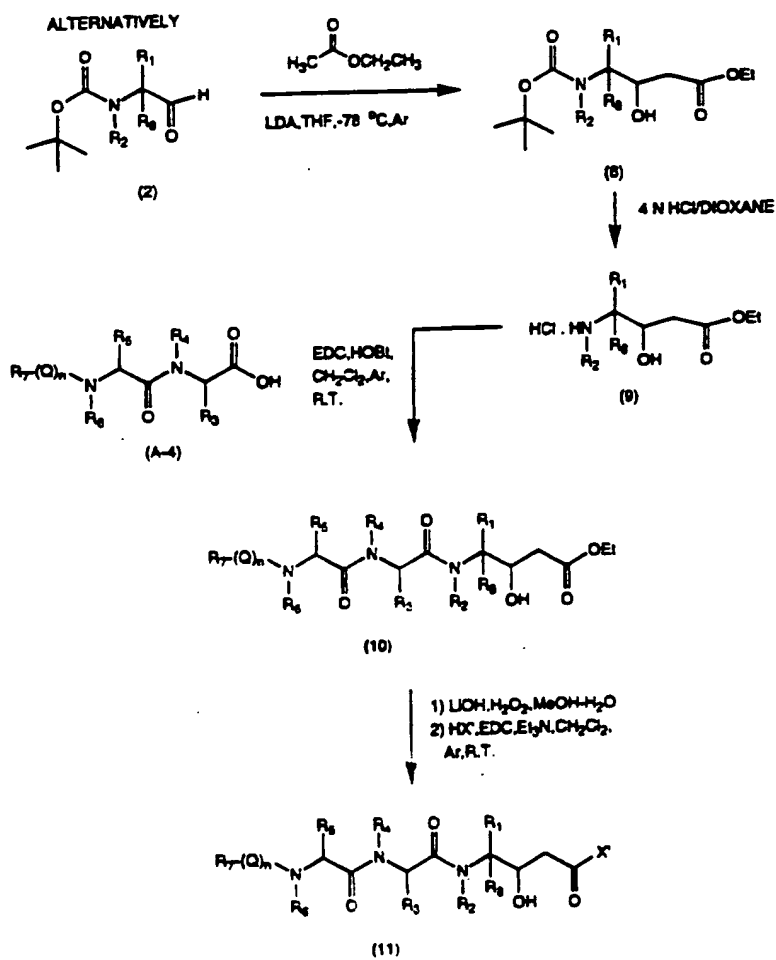
wherein Y is OR₉, SR₉, NR₉R₉ or a 5-6 ring saturated heterocycle aryl having at least one ring atom being O, S or N, and R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and (Q)_m are as previously defined.

-40-

REACTION SCHEME M



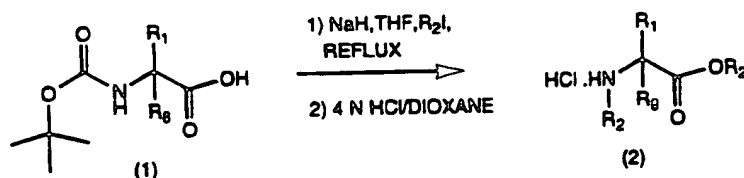
wherein $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5, \text{R}_6, \text{R}_7, \text{R}_8$ and $(\text{Q})_m$ are as defined above.
 wherein R_9 is C_{1-4} alkyl, phenyl, benzyl or hydrogen.



wherein the $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5, \text{R}_6, \text{R}_7, \text{R}_8$ and $(\text{Q})_n$ are as defined above.
 wherein R_9 is C_{1-6} alkyl, phenyl, benzyl or hydrogen and X is OR_{10} or $\text{NR}_{10}\text{R}_{11}$.

-42-

REACTION SCHEME N



wherein the R₁, R₂ and R₃ are as defined above.

Alternative methods for preparing aldehydes are depicted in Reaction Schemes A, B and C. In Scheme A, the process is initiated by coupling the protected amino acid (1) with the N-methyl-N-methoxyamine hydrochloride (2) utilizing EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide as its HCl salt) in the presence of hydroxybenzyl triazole (HOBt) and triethylamine (Et₃N); the reaction is conducted in CH₂Cl₂ at room temperature under anhydrous conditions. Upon completion of the coupling reaction, the amine-protecting group is removed by reaction with 4 N HCl in dioxane to yield compound (3). The resulting N-methyl-N-methoxyamide derivatives (sometimes referred to as a Weinreb amide) are coupled to a N-protected dipeptide (4) to produce the analogous N-methyl-N-methoxyamide intermediates (5) which are reduced to the desired aldehydes using standard reduction conditions, e.g., by the use of lithium aluminum hydride (LiAlH₄) in tetrahydrofuran (THF) at about 0°C in an inert atmosphere (Ar or N₂) under anhydrous conditions. After the reaction is complete, about 30 min, the reaction is quenched by the addition of, for example, 10% potassium hydrogen sulfate and then water.

In Reaction Scheme B, the intermediates (A-4; compound 4 prepared in Scheme A) are coupled with an ester (derived by a standard Fischer esterification of (1)) and the resulting esters (B-3) are reduced to their

-43-

corresponding alcohols (B-4) using lithium borohydride in THF at temperatures of about 0°C to room temperature; the reduction, of course, being conducted under anhydrous conditions in an inert atmosphere. The resulting alcohols (B-4) are oxidized to the desired aldehydes using the well-known Swern oxidation procedure.

In Scheme C, the esters (1) are subjected to mild hydrolysis using LiOH and hydrogen peroxide in a methanol-water solvent according to known conditions. Using the same coupling procedure as described in Scheme A1, the resulting amino acids are coupled with propane sulfide to produce the thioesters (2) which are reduced to their corresponding aldehyde by using Pd on carbon with triethylsilane under anhydrous conditions at room temperature under an inert atmosphere.

Reaction Scheme D illustrates the preparation of precursor reactants which are amenable to substitutions on the α -carbon atom; these substituents not necessarily being residues of naturally occurring α -amino acids. In this scheme, N-(diphenylmethylene)glycine ethyl ester (1) is treated with lithium bis(trimethylsilyl)amide wherein the reaction is effected in THF under an inert atmosphere at temperatures of about -78°C and the *in situ* generated base is reacted with the appropriate alkyl halide to effect a nucleophilic displacement. The so-alkylated intermediates (2) and (4) are subjected to hydrolysis to produce the amines (3) and (5) which are available for appropriate use in the construction of the desired dipeptides and tripeptides wherein R₁, R₃ or R₅ are side chains other than that of a naturally occurring α -amino acid. Similar such process may be useful in preparing compounds wherein R₂, R₄ or R₆ are an alkylated product.

Reaction Scheme E illustrates the preparation of the P₁ moiety wherein X is nitrile. The synthesis is initiated by reacting an N-protected amino acid with *iso*-butyl chloroformate in the presence of 4-methylmorpholine at -78°C under an inert atmosphere and the resulting mixed anhydride derivatives are treated with ammonia gas at -78°C to form the

-64-

corresponding amides (2). The amide is converted to its nitrile by dehydration with tosyl chloride in pyridine followed by the removal of the Boc protecting group by hydrolysis with 4 N HCl in dioxane. Following their preparation, the P_1 moieties are coupled with the appropriate P_2P_3 moiety (A-4), or with the appropriate P_2 moiety (J-5) to produce the nitriles of E-5 (or in the case of coupling with the P_2 moiety of J-5, the corresponding nitrile analogues to the aldehydes (J-7)).

Reaction Scheme F illustrates the preparation of compounds of a P_1 moiety wherein X is a C(O)W, defined as above, C_{1-6} alkyl or aralkyl. In effecting the preparations, the N-methyl-N-methoxyamide derivative (1) is treated with a lithiothiazole nucleophile generated *in situ* to produce a ketothiazole which is deprotected by hydrolysis with 4 N HCl in dioxane to obtain compounds (2) which are then coupled to the appropriate P_2P_3 moieties to obtain the derivatives of compound (3) or with the appropriate P_2 moieties (i.e., $(R_A)CH(R_B)-C(O)N(R_A)-CH_2(R_3)C(O)OH$) to obtain the desired dipeptides. Of course, the lithio derivative of thiazole may be replaced with litho derivatives of other aryl, aralkyl and alkyl moieties and by following substantially the same procedures, the corresponding di- and tri- peptides may be obtained.

Reaction Scheme G illustrates the preparation of compounds of formulae I and II wherein the X moiety is a haloketone. The reaction is initiated by reacting an R_1 -substituted nitromethane with a trifluoromethyl acetal (2) in DMF in the presence of potassium carbonate at about 60°C to yield a 1,1,1-trifluoro-2-hydroxy-3-nitro derivative (3) which are reduced with H_2 in the presence of Raney Nickel to yield the corresponding amines (4). By appropriate coupling, the alcohols of (6) may be produced which would then be subjected to Dess-Martin oxidation to produce the desired CF_3 analogues (7). By use of mono- and di-fluoromethyl analogues of formula (2) and by following substantially the same procedures, there are produced the corresponding $-CH_2F$ and $-CHF_2$ ketone analogues.

Reaction Scheme H illustrates the preparation of compounds of formulae I and II wherein the X represents an α -ketoester or α -ketoamide ($C(O)C(O)OR_D$ or $C(O)C(O)NR_D R_D$, respectively). The embodiment depicted in Reaction Scheme H is one in which R_D and R_E are the same; it is however
 5 not intended that R_D and R_E be limited to being the same and it is understood that the scheme set forth also encompasses embodiments in which R_D and R_E are not the same. Thus, such reactions are performed by replacing one of the R_D with R_E .

As set forth in Reaction Scheme H, in this process, the peptides are
 10 subjected to a modified Dakin West reaction to generate an enol ester which is subjected to a basic hydrolysis to obtain the α -keto esters (2). To transform the α -keto esters to their corresponding amides, the ketone is protected by a ketal formation by reaction with $HSCH_2CH_2SH$ in the presence of a BF_3 etherate. Treatment with ethanol at $0^\circ C$ in the presence
 15 of the appropriate amines forms the amide moiety and deprotects the ketal to form the α -keto amide (4). Alternatively, hydrolysis of intermediate (2) produces the α -keto acid (5).

Reaction Scheme I illustrates the preparation of compounds of formulae I and II wherein the X moiety is a diazomethane which may be
 20 converted to a halomethyl ketone. In this process the amine protected peptides are subjected to reaction with *iso*-butyl chloroformate in the presence of 4-methylmorpholine in CH_2Cl_2 at $-78^\circ C$. The mixed anhydride derivatives are reacted with diazomethane according to standard procedures well known in the art. If desired, the diazoketones of formulae II may be
 25 treated with the appropriate acid (e.g., HF, HCl, HBr), in pyridine to afford the desired ketohalomethyl derivatives.

Reaction Scheme J illustrates the preparation of compounds of formulae I and II wherein the amino terminus of the peptide is modified to produce compounds embraced within the scope of the $\{R_A(R_B)-CH-(Q)_n\}$ of
 30 formulae II as well as compounds which fall within the scope of $R_7-(Q)_n$ of

-46-

formulae I wherein $(Q)_n$ is $C(O)$. The scheme is initiated by the conversion of the acid (1) to its acid chloride. Treatment of the acid chloride with the appropriate chiral auxiliary (e.g., 4S,5R-(-)-4-methyl-5-phenyl-oxazolidinone) in the presence of triethylamine in CH_2Cl_2 produces the imides (2). These
5 imides are treated with lithium bis(trimethylsilyl)amide in tetrahydrofuran at $-78^\circ C$ (initially) and the resulting activated moiety, generated *in situ*, is subjected to a stereoselective alkylation using a haloelectrophile. The alkylation is completed as the mixture warms to room temperature to produce compounds (3) as pure enantiomers. Hydrolysis with lithium
10 hydroxide in hydrogen peroxide produces the acids (4) which are coupled to an amino acid (in its ester form) using the described EDC, HOBt, Et_3N coupling process, followed by hydrolysis of the ester. The hydrolyzed amino acid is subjected to another coupling reaction with an amino acid (in its ester form), and the ester of the resulting dipeptide is reduced with lithium
15 borohydride to its corresponding alcohol (6). The alcohol is converted to its aldehyde using the Swern oxidation procedure. Of course, by selecting the appropriate chiral auxiliary and substantially following the outlined procedure, other stereospecific enantiomers may be produced.

Reaction Scheme K illustrates the formation of a dipeptide wherein R_a
20 may represent an aryloxy, aralkoxy or an alkoxy in an enantiomeric pure isomer. The reaction is initiated by a two step process wherein (a) compound (1) is hydrodeaminated by treatment with $NaNO_2$ in HCl and (b) an esterification of the acid with an alkyl halide in the presence of DMF and cesium carbonate to produce compounds (2). These are treated with a
25 2,2,2-trichloroacetate derivatives in the presence of trifluoromethanesulfonic acid in CH_2Cl_2 to obtain the desired ester and the ester moiety is hydrolyzed with lithium hydroxide in peroxide and a methanol-water solvent to produce the enantiomers of formula (3). The isomers are coupled with the appropriate P_2P_1 moieties (as esters), the resulting esters

-47-

(5) are reduced to their corresponding alcohols which are then oxidized to the corresponding aldehydes (6).

Reaction Scheme L illustrates the preparation of compounds of formulae I and II wherein the X is defined by the moiety $C(O)CH_2Y$. The N-protected diazoketone derivatives of compound (1) are subjected to an addition reaction with an hydrohalic acid, preferably HCl, in pyridine to produce halomethyl derivatives (2), which are subjected to a nucleophilic displacement reaction using an activated anion of the desired Y moiety, (e.g., Y^-), to afford compounds (3). Standard hydrolysis reactions remove the N-protecting group followed by the usual coupling procedures with the desired P_2P_3 moieties (e.g., compounds A-4) to produce the desired compounds (5).

Reaction Scheme M illustrates the preparation of compounds of formulae I and II wherein the X is defined by the moieties (a) $-CH(OH)-C(O)-NR_D R_D$ and (b) $-CH(OH)-C(O)OR_D$. The embodiment depicted in Reaction Scheme M is one in which R_D and R_E are the same, it is however not intended that R_D and R_E be limited to being the same and it is understood that the scheme set forth also encompasses embodiments in which R_D and R_E are not the same. Thus, such reactions are performed by replacing one of the R_D with R_E .

As set forth in Reaction Scheme M, the process conveniently starts with the obtention of the aldehyde (2) by reducing the N-methyl-N-methoxy amide derivative (1), followed by preparation of the cyanohydrin (3) which is hydrolyzed to its free acid (4) using standard and well known reaction techniques. Coupling of the desired dipeptides (i.e., the P_2P_3 moiety) to the acid (4) is effected by the use of an activated *iso*-butyl chloroformate in the presence of 4-methylmorpholine at $-78^\circ C$ in an inert atmosphere under anhydrous conditions to afford the acid (5). The acids (5) may be esterified to its corresponding ester or may be coupled with an amine ($NR_D R_D$) to produce the desired amides (6). Of course, using substantially the same

-48-

procedure but replacing the P_2P_3 moieties of formula A-4 with the appropriate P_2 moiety, analogous dipeptides may be prepared.

Alternatively, compounds (2) may be transformed to their N-protected (preferably a Boc group) alkyl ester by reaction with ethylacetate in the presence of LDA to produce compounds (8) which are hydrolyzed with 4 N HCl in dioxane to remove the protecting group to produce the corresponding β -hydroxy ethyl esters (9). These esters are then coupled with compounds (A-4) and the resulting compounds are hydrolyzed to their β -hydroxy acids or they may be coupled to form their β -hydroxyamides of compounds (11).

Reaction Scheme N illustrates the process by which compounds of formulae I and II wherein the R_2 , R_4 , or R_6 represent an R_D moiety other than H. In essence, the procedure utilizes standard N-protection, N-alkylation - esterification and de-protection procedures such as those exemplified in the depicted schemes. Of course, although the reaction scheme depicts N-alkylation at the projected P_1 moiety, any of the P_2 and P_3 moieties may be similarly N-alkylated by appropriate selection of the starting materials followed by the coupling procedures required to construct the desired peptides of formulae I and II.

Throughout the above presentation of the methods useful for preparing the compounds herein, particularly as it relates to the foregoing reaction schemes, the full embodiment of the entire scope of the compounds (as defined in formulae I and II) was not depicted within all of the structures illustrated for each of the reactants and end-products. The state of the art is such that one of skill in the art would be able to extend these specific illustrations to embrace the implied generic teachings by the use of analogy or reasoning to prepare the desired compounds embraced within the scope of formulae I and II. For example, in Reaction Scheme A it is to be noted that the final compounds (i.e., compounds (6) of that reaction scheme) are tripeptides. It can be seen that by substituting the reactant (4) with the appropriate α -amino acid, and by following the teachings herein, dipeptides

-49-

would result. Similarly one of skill in the art could utilize the final product of Reaction Scheme D in preparing any of the compounds of formulae I and II bearing the R₁ side chain functionality, which is other than a residue of a naturally occurring α -amino acid. Similarly, in Reaction Scheme F, the preparation of a thiazole derivative is achieved by coupling the N-methoxy-N-methylamide derivative of a precursor for preparing a depicted tripeptide (3). A dipeptide bearing a thiazole derivative could be prepared by the application of the analogy reasoning possessed by a person of skill in the art. Similarly, lithio derivative of another heterocycle in which X is C(O)aryl and the aryl moiety is other than a thiazole embraced within the scope of the compounds herein could be prepared.

Thus, the scope of those compounds preparable by the methods of the foregoing reaction schemes is not limited to the specific compounds depicted but rather to those compounds defined by formulae I and II using the teachings already available in the art and which are exemplified to illustrate such teachings.

2. Procedures to effect the reaction schemes

The construction of the tri- and dipeptide analogs of Formulae I and II may be effected using procedures and techniques well known in the art and described herein. Many of the necessary starting materials and the reactants utilized are known and may also be commercially available. In those instances in which they are not generally available, they may readily be generated by analogous use of known chemical processes and techniques readily available in the scientific and patent literature or as described herein.

As the reaction schemes depicted herein (Schemes A-N) extensively utilize coupling and oxidation procedures, the following elaborates a variety of the procedures that may be functional alternatives to those specifically mentioned within the depicted schemes.

As a preferred oxidation procedure, the Swern oxidation is effected by reacting 2 to 10 equivalents of dimethyl sulfoxide (DMSO) with about 1

-50-

to 6 equivalents of trifluoroacetic anhydride $[(CF_3CO)_2]$ or oxalyl chloride $[(COCl)_2]$. The reactants are dissolved in an inert solvent, e.g., methylene chloride (CH_2Cl_2), the reactor is under an inert atmosphere under anhydrous conditions at temperatures of about $-80^\circ C$ to $-50^\circ C$ to form an *in situ* sulfonium adduct to which is added about 1 equivalent of the alcohols (e.g., B-4). Preferably, the alcohols are dissolved in an inert solvent, e.g., CH_2Cl_2 or minimum amounts of DMSO, and the reaction mixture is allowed to warm to about $-50^\circ C$ (for about 10-20 minutes) and then the reaction is completed by adding 3 to 10 equivalents of a tertiary amine, e.g., triethylamine, *N*-methyl morpholine, etc. Following oxidation, the desired intermediates are isolated and are ready for the next step in the reaction sequence.

A modified Jones oxidation procedure may conveniently be effected by reacting the alcohols with pyridinium dichromate by contacting the reactants in a water-trapping sieve powder, (e.g., a grounded 3 Angström molecular sieve) in the presence of glacial acetic acid at about $5^\circ C$ to $50^\circ C$, preferably at room temperature.

Alternatively, 1 to 5 equivalents of a chromic anhydride-pyridine complex [i.e., a Sarett reagent prepared *in situ* (see, e.g., Fieser and Fieser "Reagents for Organic Synthesis" Vol. 1, pp. 145 and Sarett, *et al.*, *J.A.C.S.* 25, 422 (1953))] that is prepared *in situ* in an inert solvent (e.g., CH_2Cl_2) in an inert atmosphere under anhydrous conditions at about $0^\circ C$ to $50^\circ C$ to which complex is added 1 equivalent of the alcohols allowing the reactants to interact for about 1 to 15 hours, followed by the isolation of the desired product.

Another alternative process for the converting of alcohols to the desired ketones is an oxidation reaction that employs periodane [i.e., 1,1,1-triacetoxy-1,1-dihydro, 1,2-benzoxido 3-(1-H)-one (see Dess Martin, *J. Org. Chem.* 48, 4155, (1983))]. This oxidation is effected by contacting 1 equivalent of the alcohols with 1 to 5 equivalents of periodane (preferably 1.5 equivalents) in suspension in an inert solvent (e.g., CH_2Cl_2) under an

-51-

inert atmosphere (preferably nitrogen) under anhydrous conditions at about 0°C to 50°C (preferably room temperature), and allowing the reactants to interact for about 1 to 48 hours.

A solid phase sequential coupling procedure can be performed using established methods such as use of an automated peptide synthesizer. In this procedure, an amino protected amino acid is bound to a resin support at its carboxyl terminus, the protected amine is deprotected where the peptide linkage is desired, the amino group neutralized with a base and the next amino protected amino acid in the desired sequence is coupled in a peptide linkage. The deprotection, neutralization and coupling steps are repeated until the desired peptide is synthesized. Thus, the compounds provided herein can be synthesized from their carboxyl terminal end to their amino terminal end. The amino protected amino acid can be a conventional amino acid, a derivative or isomer thereof, or a spacer group. The resin support employed can be any suitable resin conventionally employed in the art for the solid phase preparation of polypeptides. The preferred resin is polystyrene which has been cross-linked with from about 0.5 to about 3% divinyl benzene, which has been either benzhydrylamidated, chloromethylated or hydroxymethylated to provide sites for amide or ester formation with the initially introduced amino protected amino acid.

An example of a hydroxymethyl resin is described by Bodansky *et al.* [*Chem. Ind.* (London) 38, 1597-98 (1966)]. The preparation of chloromethyl and benzhydrylamine resins are described by Stewart *et al.* [*"Solid Phase Peptide Synthesis,"* 2nd Edition, Pierce Chemical Co., Rockford, Illinois (1984). Chapter 2, pp. 54-55]. Many of these resins are available commercially. In general, the amino protected amino acid which is desired on the carboxyl-terminal end of the peptide is bound to the resin using standard procedures and practices as are well known and appreciated in the art. For example, the amino protected amino acid can be bound to the resin by the procedure of Gisin [*Helv. Chem. Acta*, 56, 1476 (1973)].

-52-

When it is desired to use a resin containing a benzhydrylamine moiety as the resin binding site an amino protected amino acid is coupled to the resin through an amide linkage between its α -carboxylic acid and the amino moiety of the resin. The coupling is effected using standard coupling procedures as described below. Many resin-bound amino acids are available commercially.

The α -amino protecting group employed with each amino acid introduced into the polypeptide sequence may be any such protecting group known in the art. Among the classes of amino protecting groups contemplated are: (1) acyl type protecting groups such as formyl, trifluoroacetyl, phthalyl, *p*-toluenesulfonyl (tosyl), benzenesulfonyl, nitrophenylsulfonyl, tritylsulfonyl, *p*-nitrophenoxycarbonyl, and α -chlorobutyryl; (2) aromatic urethane type protecting groups such as benzyloxycarbonyl and substituted benzyloxycarbonyls such as *p*-chlorobenzyloxycarbonyl, *p*-methoxybenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, 1-(*p*-biphenyl)-1-methylethoxycarbonyl, α,α -dimethyl-3,5-dimethoxybenzyloxycarbonyl, and benzhydryloxycarbonyl; (3) aliphatic urethane protecting groups such as *t*-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropylloxycarbonyl, ethoxycarbonyl, and allyloxycarbonyl; (4) cycloalkyl urethane type protecting groups such as cyclopentylloxycarbonyl, adamantylloxycarbonyl, and cyclohexylloxycarbonyl; (5) thiourethane type protecting groups such as phenylthiocarbonyl; (6) alkyl type protecting groups such as triphenylmethyl (trityl) and benzyl (Bn); (7) trialkylsilane protecting groups such as trimethylsilane, 4-[(4-chlorophenyl) sulfonylamino] carbonyl, and 4-[(4-bromophenyl) sulfonylamino] carbonyl. The preferred α -amino protecting group is *t*-butyloxycarbonyl (Boc); its use as an α -amino protecting group for amino acids is well known to those of skill in the art ([see, e.g., by Bodansky *et al.* in "The Practice of Peptide Synthesis," Springer-Verlag, Berlin (1984), p.20).

-53-

Following the coupling of the amino protected amino acid to the resin support, the α -amino protecting group may be removed using any suitable procedure such as by using trifluoroacetic acid, trifluoroacetic acid in CH_2Cl_2 , or HCl in dioxane. The deprotection is carried out at a temperature of
5 between 0° C and room temperature. Other standard cleaving reagents may be used for removal of specific amino protecting groups under conditions well known and appreciated in the art.

After removal and neutralization of the α -amino protecting group, the next desired amino-protected amino acid is coupled through a peptide
10 linkage. This deprotection, neutralization and coupling procedure is repeated until a peptide of the desired sequence is obtained. Alternatively, multiple amino acid groups may be coupled by the solution method prior to coupling with the resin supported amino acid sequence.

The selection and use of an appropriate coupling reagent is within the
15 skill of the skilled artisan. Particularly suitable coupling reagents where the amino acid to be added is Gln, Asn, or Arg include N,N-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The use of these reagents prevents nitrile and lactam formation. Other coupling agents are (1) other carbodiimides (e.g., N-ethyl-N'-(γ -dimethylaminopropylcarbodiimide);
20 (2) ketenimines; (3) isoxazolium salts (e.g., N-ethyl-5-phenylisoxazolium-3-sulfonate); (4) monocyclic nitrogen-containing heterocyclic amides of aromatic character containing one through four nitrogens in the ring such as imidazolides, pyrazolides, and 1,2,4-triazolides (specific heterocyclic amides that are useful include N,N-carbonyldiimidazole and N,N-carbonyl-di-1,2,4-
25 triazole); (5) alkoxylated acetylene (e.g., ethoxyacetylene); (6) reagents which form a mixed anhydride with the carboxyl moiety of the amino acid (e.g., ethyl chloroformate and *iso*-butyl chloroformate) or the symmetrical anhydride of the amino acid to be coupled (e.g., Boc-Ala-O-Ala-Boc);
30 (7) nitrogen-containing heterocyclic compounds having a hydroxyl group on one ring nitrogen (e.g., N-hydroxyphthalimide, N-hydroxysuccinimide, and

-54-

1-hydroxybenzotriazole). Other activating reagents and their use in peptide coupling are described by Kapoor [*J. Pharm. Sci.*, **59**, 1-27 (1970)]. Use of the symmetrical anhydride as the coupling agent is the generally preferred amino acid coupling method herein.

5 The preferred coupling method for Gln, Asn and Arg is to react the protected amino acid, or derivatives or isomers thereof, with N,N-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (1:1) in N,N-dimethylformamide (DMF) in the presence of the resin or resin-bound amino acid or peptide. The preferred coupling method for other amino acids
10 involves reacting the protected amino acid, or derivative or isomer thereof, with N,N-dicyclohexylcarbodiimide in CH_2Cl_2 to form the symmetrical anhydride. The symmetrical anhydride is then introduced into the solid phase reactor containing the resin or resin-bound amino acid or peptide, and the coupling is carried out in a medium of DMF, or CH_2Cl_2 , or DMF: CH_2Cl_2
15 (1:1). A medium of DMF is preferred. The success of the coupling reaction at each stage of the synthesis is monitored by a ninhydrin test as described by Kaiser *et al.* [*Analyt. Biochem.* **34**, 595 (1970)]. In cases where incomplete coupling occurs, the coupling procedure is repeated. If the coupling is still incomplete, the deprotected amine is capped with a suitable
20 capping reagent to prevent its continued synthesis. Suitable capping reagents and the use thereof are well known and appreciated in the art. Examples of suitable capping reagents are acetic anhydride and acetylimidazole as described by Stewart *et al.* ["Solid Phase Peptide Synthesis," 2nd Ed., Pierce Chemical Co., Rockford, Ill. (1984), Chapter 2, p.73].

25 After the desired amino acid sequence has been obtained, the peptide is cleaved from the resin. This can be effected by procedures which are well known and appreciated in the art, such as by hydrolysis of the ester or amide linkage to the resin. It is preferred to cleave the peptide from the benzhydrylamine resin with a solution of dimethyl sulfide, *p*-cresol,
30 thiocresol, or anisole in anhydrous hydrogen fluoride. The cleavage reaction

-55-

is preferably carried out at temperatures between about 0°C and about room temperature, and is allowed to continue preferably from between about 5 minutes to about 5 hours.

- As is known in the art of solid phase peptide synthesis, many of the
- 5 amino acids bear side chain functionalities requiring protection during the preparation of the peptide. The selection and use of an appropriate protecting group for these side chain functionalities is within the ability of those skilled in the art and will depend upon the amino acid to be protected and the presence of other protected amino acid residues in the peptide.
- 10 The selection of such a side chain protection group is critical in that it must not be removed during the deprotection and coupling steps of the synthesis. For example, when Boc is used as the α -amino protecting group, the following side chain protecting groups are suitable: *p*-toluenesulfonyl (tosyl) moieties can be used to protect the amino side chains of amino acids such
- 15 as Lys and Arg; *p*-methylbenzyl, acetamidomethyl, benzyl (Bn), or *i*-butylsulfonyl moieties can be used to protect the sulfide-containing side chains of amino acids such as cysteine, homocysteine, penicillamine and the like or derivatives thereof; benzyl or cyclohexyl ester moieties can be used to protect carboxylic acid side chains of amino acids such as Asp, Glu; a
- 20 benzyl ether can be used to protect the hydroxyl-containing side chains of amino acids such as Ser and Thr; and a 2-bromocarbobenzoxy (2Br-Cbz) moiety can be used to protect the hydroxyl-containing side chains of amino acids such as Tyr. These side chain protecting groups are added and removed according to standard practices and procedures well known in the
- 25 art. It is preferred to deprotect these side chain protecting groups with a solution of anisole in anhydrous hydrogen fluoride (1:10). Typically, deprotection of side chain protecting groups is performed after the peptide chain synthesis is complete but these groups can alternatively be removed at any other appropriate time. It is preferred to deprotect these side chains
- 30 at the same time as the peptide is cleaved from the resin.

-56-

The compounds are then isolated and purified by standard techniques. The desired amino acids, derivatives and isomers thereof can be obtained commercially or can be synthesized according to standard practices and procedures well known in the art.

- 5 C. Identification of preferred compounds using assays that identify compounds that modulate processing of amyloid precursor protein (APP)

Compounds provided herein modulate the processing of proteins, such as amyloid precursor protein (APP), involved in neurodegenerative diseases.

- 10 The ability of compounds to modulate processing of APP can be demonstrated in a variety of ways. For example, compounds can be evaluated for the ability to modulate generation of A β or α -sAPP.

1. In vitro assays

- Compounds provided herein yield a positive result in one or more in vitro assays that assess the effects of test compounds on processing of APP. In particular, in vitro assay systems for identifying such compounds are provided herein. These assays evaluate the effects of a test compound on processing of APP and use cultured human glioblastoma cell lines that have been transfected with DNA encoding either a wild-type 695 amino acid isoform of APP or a mutein of the 695 amino acid isoform of APP that contains changes (in this case two or three amino acid changes have been made) that appear to make the molecule more susceptible to proteolytic cleavage that results in increased production of A β [see, e.g., Mullan et al. (1992) Nature Genet. 1:345-347].

- 25 In performing these assays, a test compound is added to the culture medium and, after a selected period of time, the culture medium and/or cell lysates are analyzed using immunochemical assays to detect the relative amounts of A β , total soluble APP (sAPP), a portion of sAPP designated α -sAPP, and C-terminal fragments of APP. In particular, the culture medium and cell lysates are analyzed by immunoblotting coupled with laser scanning
- 30

-57-

densitometry and ELISAs using several different antibodies. A positive test occurs when: (1) there is a decrease in the ~4-kDa amyloid β -protein ($A\beta$) in the medium relative to control cultures (4-kDa assay); and/or (2) the relative amount of total sAPP in the medium increases (e.g., relative to the amount of total sAPP in medium from appropriate control cells such as cells not exposed to test compound); and/or (3) there is a decrease in the amount of C-terminal amyloidogenic fragments larger than 9 kDa and smaller than 22 kDa in the cell lysate as a result of differential processing; and/or (4) there is an increase in the amount of α -sAPP in the medium relative to control cultures. Control cultures can be cultures that have not been contacted with the compound. The $A\beta$ assay is done using cells (e.g., HGB 717/Swed) that have been transfected with DNA encoding the mutant APP; the other assays are performed using cells, such as HGB695 cells, that have been transfected with DNA encoding a wild-type APP.

Preferred compounds have activity that is at least 2-fold, preferably 5-fold, most preferably 10-fold greater activity than N-Acetyl-leucyl-leucyl-norleucinal [see, e.g., EP 0 504 938 A2; and Sherwood *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:3353-3357] in at least one, preferably the $A\beta$ assay, of these assays.

2. The amount of α -sAPP and the ratio of α -sAPP to total sAPP in cerebrospinal fluid (CSF) as an indicator of Alzheimer's Disease (AD) and the effectiveness of therapeutic intervention

The relative amount of α -sAPP and the ratio of α -sAPP to total sAPP in CSF are shown herein to be useful markers in the detection of neurodegenerative disorders characterized by cerebral deposition of amyloid (e.g., AD) and in monitoring the progression of such disease. Furthermore, assay systems incorporating these markers can be used in monitoring therapeutic intervention of these diseases.

As shown in EXAMPLE 32, the amount of α -sAPP and the ratio of α -sAPP to total sAPP in CSF samples can be used as an indicator of

-58-

Alzheimer's Disease and other neurodegenerative disorders. For purposes herein, this amount and/or the ratio can also be used to assess the effectiveness of compounds provided herein in treating Alzheimer's Disease and neurodegenerative disorders.

5 It has been found that patients with suspected Alzheimer's disease (as diagnosed by other indicia, or confirmed by autopsy) have a statistically significant lower ratio of α -sAPP to total sAPP in CSF and also have statistically significant lower levels of α -sAPP. Therefore, by comparison with non-Alzheimer's disease controls or by existence of a ratio lower than
10 a predetermined standard, based, for example, on averages in samples from large numbers of unafflicted individuals, or an amount of α -sAPP lower than a predetermined standard, Alzheimer's disease or, depending upon other indications, another neurodegenerative disease is indicated.

Compounds, such as those provided herein, that alter this ratio or the
15 level of α -sAPP closer to that of individuals who do not have a neurodegenerative disorder characterized by the cerebral deposition of amyloid are considered useful for treating these disorders.

3. In vivo assays

The ability of compounds to modulate processing of APP can also be
20 evaluated in vivo (see, e.g., Kowall et al. (1991) Proc. Natl. Acad. Sci. U.S.A. 88:7247-7251). Compounds can be administered through a canula implanted in the cranium of a rodent or other suitable test animal (see, e.g., Lamb et al. (1993) Nature Genet. 5:22-29; Pearson et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:10578-10582). After a predetermined period of
25 administration the rodents are sacrificed. Selected brain regions, e.g., hippocampi, or CSF are evaluated in immunoblot assays or other suitable assays to determine the relative level of α -sAPP, or the ratio of α -sAPP to total sAPP, and amyloidogenic C-terminal fragments of APP compared to untreated control animals. Compounds that result in relative increases in the

-59-

amount and/or ratio of α -sAPP are selected, as are those which decrease the amount of amyloidogenic C-terminal fragments of APP.

D. Formulation of pharmaceutical compositions

Compositions are provided that contain therapeutically effective amounts of the compounds of formulae (I) and (II). The compounds are preferably formulated into suitable pharmaceutical preparations such as tablets, capsules or elixirs, for oral administration or in sterile solutions or suspensions for parenteral administration, as well as transdermal patch preparation. Typically the compounds described above are formulated into pharmaceutical compositions using techniques and procedures well known in the art.

About 10 to 500 mg of a compound or mixture of compounds for Formulae I and II or a physiologically acceptable salt is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in those compositions or preparations is such that a suitable dosage in the range indicated is obtained.

To prepare compositions, one or more compounds of formulae (I) and (II) are mixed with a suitable pharmaceutically acceptable carrier. Upon mixing or addition of the compound(s), the resulting mixture may be a solution, suspension, emulsion or the like. Liposomal suspensions may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the disease, disorder or condition treated and may be empirically determined.

-60-

Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration.

In addition, the active materials can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action or have other action. The compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients.

In instances in which the compounds exhibit insufficient solubility, methods for solubilizing compounds may be used. Such methods are known to those of skill in this art, and include, but are not limited to, using cosolvents, such as dimethylsulfoxide (DMSO), using surfactants, such as tween, Creamaphor (Sigma Chemical Company) or polyethylene glycol/ethanol solution (80% v/v:20% v/v) or dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as salts of the compounds or prodrugs of the compounds may also be used in formulating effective pharmaceutical compositions.

The concentrations of the compounds that are effective for delivery of an amount, upon administration, that ameliorates the symptoms of the disorder for which the compounds are administered. Typically, the compositions are formulated for single dosage administration.

The compounds of formulae (I) and (II) may be prepared with carriers that protect them against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems.

The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the

-61-

compounds in known in vitro and in vivo model systems for the treated disorder.

The compositions can be enclosed in ampules, disposable syringes or multiple or single dose vials made of glass, plastic or other suitable material.

- 5 Such enclosed compositions can be provided in kits.

The concentration of active compound in the drug composition will depend on absorption, inactivation and excretion rates of the active compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

- 10 The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test data. It is
15 to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions,
20 and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

- If oral administration is desired, the compound should be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that
25 maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

- Oral compositions will generally include an inert diluent or an edible carrier and may be comprised into tablets or enclosed in gelatin capsules.
30 For the purpose of oral therapeutic administration, the active compound or

-62-

compounds can be incorporated with excipients and used in the form of tablets, capsules or troches. Pharmaceutically compatible binding agents and adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder, such as, but not limited to, gum tragacanth, acacia, corn starch or gelatin; an excipient such as microcrystalline cellulose, starch and lactose, a disintegrating agent such as, but not limited to, alginic acid and corn starch; a lubricant such as, but not limited to, magnesium stearate; a glidant, such as, but not limited to, colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; and a flavoring agent such as peppermint, methyl salicylate, and fruit flavoring.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active materials can also be mixed with other active materials which do not impair the desired action, or with materials that supplement the desired action.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent, such as water for injection, saline solution, fixed oil, a naturally occurring vegetable oil like sesame oil, coconut oil, peanut oil, cottonseed oil, etc. or a synthetic fatty vehicle like ethyl oleate or the like, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens;

-63-

antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. Parenteral preparations can be enclosed in
5 ampules, disposable syringes or multiple dose vials made of glass, plastic or other suitable material. Buffers, preservatives, antioxidants and the like can be incorporated as required.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing
10 thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof. Liposomal suspensions, including tissue-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be
15 prepared as described in U.S. Patent No. 4,522,811.

The active compounds may be prepared with carriers that protect the compound against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release
20 formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as collagen, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others. Methods for preparation of such formulations are known to those skilled in the art.

The compounds may be formulated for local or topical application,
25 such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Such solutions, may be formulated as 0.01% - 100% (weight to volume) isotonic solutions, pH about 5-7, with appropriate salts. The compounds may be formulated as

-64-

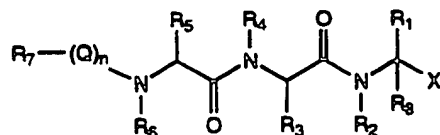
aeorsols for topical application, such as by inhalation [see, e.g., U.S. Patent Nos. 4,044,126, 4,414,209, and 4,364,923].

Finally, the compounds may be packaged as articles of manufacture containing packaging material, an acceptable composition containing a compound of formulae (I) and (II) provided herein, which is effective for treating the particular disorder, and a label that indicates that the compound or salt thereof is used for treating the disorder.

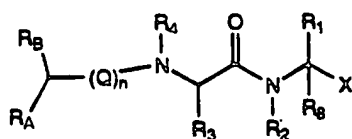
E. Methods of use

The compounds for use in the the methods herein have the formulae

(I) and (II):



I



II

or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof in which

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi), or (vii), as described above.

These compounds have pharmacological utility and also utility as reagents. It is recognized in this art that compounds that exhibit activities

-65-

in assays that assess the ability of the compounds to alter or modulate the activity of proteins associated with the deposition of cerebral amyloid, are pharmacologically useful and potentially therapeutically useful in the treatment of disorders that involve such deposition.

5 The dose ranges, which can be established empirically, for use in the treatment of disease states will depend upon the etiology, nature, and severity of the disease state as well as such other factors as determined by the attending physician. The broad range for effective treatment is about 0.01 to 10 mg per kilogram (kg) of body weight per day. The preferred
10 range is about 0.1 to 10 mg/kg of body weight per day.

The active compounds can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid, semi-liquid or solid form and are formulated in a manner suitable for each route of administration. Preferred
15 modes of administration include oral and parenteral modes of administration.

Since, it is feasible to measure the presence of, and over the course of time, to determine the rate of increase of those protein segments believed to be a critical factor influencing the formation of amyloid plaques located in the brain (see, e.g., U.S. Patent No. 5,270,165, and the CSF assay
20 provided herein, described above and in the EXAMPLES), dosages can be empirically determined by the physician. As the technique involves the use of cerebrospinal fluids, such techniques, and other equivalently functioning procedures, will be useful to the attending physician in determining the need to modify the dosage for individual patients.

25 In treating these disease states, it is sufficient to start treating the patient as soon as the attending physician makes a diagnosis that the patient is suffering from one of these diseases. Thus, although the progress of treatment of the patient may be monitored by the measurements of those biological factors which characterize the diseases, it is not necessary to so-
30 valuate such characteristics before treatment. Rather it is within the

-66-

providence of the attending physician to determine when it is in the best interest of the patient to start treatment. Therefore, patients showing increased probabilities of the disease state, (e.g., by carrying known familial genetic markers that increase the probability of the incidence of neurodegenerative diseases as well as the patient's general behavioral characteristics and other indicia of these diseases) can be treated by the methods and with the compositions provided herein.

1. Treatment of neurodegenerative diseases

Amyloid plaques are believed to accompany and/or to be involved in the process responsible for the development and progression of certain neurodegenerative disease states. Without being bound by any theory of action, it is believed that the compounds provided herein modulate the generation of amyloidogenic peptides to effectuate a beneficial result. Without any intent to limit or restrict the compounds and methods provided herein to any specific mechanism of action for the end-use applications, it is believed that the compounds effectuate a modulation of the processing of the amyloid precursor protein (APP), the progenitor of the deposited amyloidogenic A β peptides (39 to 43 amino acid residues) found in senile plaques in the brains of patients diagnosed with, for example, Alzheimer's disease. Thus, the compounds provided herein are useful in the treatment of such neurodegenerative disease states in which such amyloid plaques accumulate or are implicated in the etiology thereof, including, but not limited to: Alzheimer's disease, cognition deficits, Down's Syndrome, Parkinson's disease, cerebral hemorrhage with amyloidosis, dementia pugilistica, head trauma and in the treatment of conditions characterized by a degradation of the neuronal cytoskeleton resulting from a thrombolytic or hemorrhagic stroke.

For example, it is believed that the compounds can be used in the treatment of Alzheimer's patients through the modulation of APP processing to effectuate a beneficial result by: (a) decreasing the formation of A β ; (b)

-67-

modulating the generation of a mutually exclusive, alternative-processed form of APP that precludes A β formation (α -sAPP); and/or, (c) modulating the generation of partially processed C-terminal A β -containing amyloidogenic peptides.

5 In addition, these compounds may also beneficially modulate neurodegenerative abnormalities not thought to be associated with amyloid plaques, such as stroke, by beneficially affecting the rate of degeneration of the neuronal cytoskeleton that occurs as a result of thrombolytic or hemorrhagic stroke.

10 It is believed that the treatment of patients with such disorders with these compounds will result in a beneficial modulation of the causative factors involved in neurodegenerative disease states and will result in an enhanced lifestyle as well as to delay or obviate the need to institutionalize these patients.

15 The compounds can be administered to patients in need of such treatment in a dosage range of 0.01-10 mg per kg of body weight per day, and can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid, semi-liquid or solid form and are formulated in a manner suitable for
20 each route of administration. As stated above, the dose will vary depending on severity of disease, weight of patient and other factors which a person skilled in the art will recognize.

Patients include those with a neurodegenerative disease, including but not limited to Alzheimer's disease, cognition deficits, Down's Syndrome,
25 Parkinson's disease, cerebral hemorrhage with amyloidosis, dementia pugilistica, and head trauma. Treatment is effected by administering to such patient a therapeutically effective amount of a compound of the formulae (I) and (II) defined as above. Particularly preferred for use in these methods are the compounds of formulae (I) and (II) but with the proviso
30 that, when the compounds have formula (I): (1) at least n of the amino

-68-

acid residues in the resulting tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R_1 , R_3 and R_5 is not a side chain of a naturally-occurring amino acid; and (2) when X is an aldehyde, the non-naturally occurring amino acid (or side chain thereof) is other than norleucine or norvaline, and when the compounds have formula (II) and X is an aldehyde, R_1 is not norleucine or norvaline.

In some embodiments the compounds of formula (II) are also selected such that: (1) at least one of the amino acid residues in the resulting di-peptide is a non-naturally-occurring α -amino acid or at least one of the R_1 and R_3 is not a side chain of a naturally-occurring amino acid; and (2) when X is an aldehyde, the non-naturally occurring amino acid (or side chain thereof) is other than norleucine or norvaline.

In certain preferred embodiments, the compounds have formulae I or II, particularly formula I, as defined above, but with the proviso that: (1) at least one of the amino acid residues in the resulting tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R_1 , R_3 and R_5 is not a side chain of a naturally-occurring amino acid; and (2) when R_1 is the side chain from a non-naturally occurring amino acid, it is not the side chain of norleucine or norvaline.

In other preferred embodiments, the compounds have formulae I or II, particularly formula I, as defined above, but with the proviso that: (1) at least one of the amino acid residues in the resulting di or tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R_1 , R_3 and R_5 is not a side chain of a naturally-occurring amino acid; and (2) none of R_1 , R_3 and R_5 is the side chain of norleucine or norvaline.

2. Treatment of diseases characterized by degeneration of the cytoskeleton

Also provided are methods of treating a patient suffering from a disease state characterized by the degeneration of the cytoskeleton arising from a thrombolytic or hemorrhagic stroke by administering a therapeutically

effective amount of a compound of the formulae (I) and (II), particularly the compounds of formulae (I), with the proviso that when the compounds have formula (I): (1) at least one of the amino acid residues in the resulting di or tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R₁, R₃ and R₅ is not a side chain of a naturally-occurring amino acid; and (2) when X is an aldehyde, the non-naturally occurring amino acid (or side chain thereof) is other than norleucine or norvaline, and when the compounds have formula (II) and X is an aldehyde, R₁ cannot be norleucine or norvaline.

In certain preferred embodiments, the compounds for use in this method of treatment have formulae I or II, as defined above, but with the proviso that when the compounds have formula (I): (1) at least one of the amino acid residues in the resulting di or tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R₁, R₃ and R₅ is not a side chain of a naturally-occurring amino acid; and (2) when R₁ is the side chain from a non-naturally occurring amino acid, it is not the side chain of norleucine or norvaline, and when the compounds have formula (II) and X is an aldehyde, R₁ cannot be norleucine or norvaline.

In some embodiments the compounds of formula (II) are also selected such that: (1) at least one of the amino acid residues in the resulting di-peptide is a non-naturally-occurring α -amino acid or at least one of the R₁ and R₃ is not a side chain of a naturally-occurring amino acid; and (2) when X is an aldehyde, the non-naturally occurring amino acid (or side chain thereof) is other than norleucine or norvaline.

In other preferred embodiments, the compounds have formulae I or II, particularly formula I, as defined above, but with the proviso that: (1) at least one of the amino acid residues in the resulting di or tri-peptide is a non-naturally-occurring α -amino acid or at least one of the R₁, R₃ and R₅ is not a side chain of a naturally-occurring amino acid; and (2) none of R₁, R₃ and R₅ is the side chain of norleucine or norvaline.

-70-

3. Protease inhibition

Compounds provided herein have activity as inhibitors of proteases, such cysteine proteases, including calpain. It is believed by those of skill in this art that excessive activation of the Ca^{2+} -dependent protease calpain plays a role in the pathology of a variety of disorders, including cerebral ischaemia, cataract, myocardial ischaemia, muscular dystrophy and platelet aggregation. Thus, compounds that have activity as calpain inhibitors are considered by those of skill in this art to be useful [see, e.g., U.S. Patent No. 5,081,284, Sherwood et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:3353-3357]. Assays that measure the anti-calpain activity of selected compounds are known to those of skill in the art (see, e.g., U.S. Patent No. 5,081,284). Activities of inhibitors in such in vitro assays at concentrations (IC_{50}) in the nanomolar range or lower are indicative of therapeutic activity. Such compounds also have utility in the purification of proteinases, such as cysteine proteases, on affinity columns of these compounds (see, U.S. Patent No. 5,081,284). Also, calpain inhibitors, such as N-acetyl-leucyl-leucyl-norleucinal [see, e.g., EP 0 504 938 A2; and Sherwood et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:3353-3357], which is commercially available, are used as reagents in the study of protein trafficking and other cellular processes [see, e.g., Sharma et al. (1992) J. Biol. Chem. 267:5731-5734]. Finally, inhibitors of cysteine proteases strongly inhibit the growth of *Plasmodium falciparum* and *Schistosoma mansoni* [see, e.g., Scheibel et al. (1984) Protease inhibitors and antimalarial effects. In: Malaria and the Red Cell, Progress in Clinical and Biological Research, Alan R. Liss, Inc., NY, pp. 131-142].

4. Identification and/or isolation of proteases

Using standard protein purification techniques known in the art [see e.g., Nicholson et al. Nature 376:37-43 (1995)], the compounds can be used as affinity ligands in isolation of proteases. For example, a compound may be linked to a support, such as activated agarose, CNBr Sepharose,

-71-

streptavidin agarose or resin such as Affi-gel, covalently or by other linkage, through the $R_7-(Q)_n$ or $(R_8)-CH(R_A)-(Q)_n$ moieties. In coupling the compound to the support in this manner, the remainder of the compound molecule remains available to interact with protease proteins contained in a sample

5 passed through a column containing the compound-coupled support material. The resin is preequilibrated with a suitable buffer prior to adding the composition containing the protease to be isolated, i.e., typically a cell lysate. Interaction of the immobilized compound and protease effects separation of the protease from the other constituents of the lysate which

10 is followed by subsequent isolation of the protease through elution from the column. Preparation of the cell lysate and resin and elution of the column may be performed by modification of standard protocols known to those of skill in the art.

Compounds particularly intended for use in isolation of proteins, such

15 as specific proteases, are those displaying the most significant activity in modulating the processing of APP (determined, for example, as described in Example 31 to identify compounds with activity at lower concentrations, e.g., $IC_{50} < 50 \mu M$). These compounds may be used in isolating proteins such as secretases, including β and γ secretases, which may be involved in

20 generating fragments of APP. β and γ secretase refer to the activities associated with the cleavage of APP that gives rise to the $A\beta$ peptide.

Compounds provided herein in which the $R_7-(Q)_n$ or $(R_8)-CH(R_A)-(Q)_n$ moiety is a chemical labeling/linking group are also useful in isolating and/or detecting proteases. The labeling groups contain chemical linkers whereby

25 the label portion of the group is directly attached to the peptide compounds through the nitrogen of formulae I and II that is linked to $(Q)_n$. [See Nicholson et al., supra, for exemplary methods of linking a chemical labeling group to a peptide.] Chemical labels are well known to those skilled in the art. Biotin, a commonly used chemical label, bonded to caproic acid is an

30 example of one such chemical labeling group incorporated into compounds

-72-

of formulae I and II as $R_7-(Q)_n$ or $(R_8)-CH(R_A)-(Q)_n$. Compounds containing a biotin label can be used to detect proteases with which they interact in a sample (e.g., a cell lysate or a fraction thereof) containing the protease. The compound-protease complex is detected by exposing the sample to a biotin binding partner, such as streptavidin, which in turn is bound to a signal-generating moiety such as horse radish peroxidase (HRP). Addition of peroxidase to the sample yields a signal detected spectrophotometrically. A biotin-labeled compound provided herein can also be used to isolate proteases that interact with the compound. For example, the compound can be bound to a streptavidin-agarose resin and used as an affinity resin. Additionally, compounds in which the $R_7-(Q)_n$ or $(R_8)-CH(R_A)-(Q)_n$ moiety is a chemical linking group are also useful in isolating proteases through direct coupling of the compound to a resin through, e.g., a primary amine of the linking group. Preferred linking groups include 6-amino caproic acid and amino decanoic acid.

5. Identification of protease inhibitors and modulators of APP processing

The compounds provided herein as chromogenic or fluorogenic substrates wherein X is a chromophore or fluorophore are useful in identifying protease inhibitors. These compounds are readily prepared by using methods of synthesizing peptides of formulae I and II as described herein (see, for example, reaction schemes A-N and Examples 1-30). For example, to prepare a chromogenic substrate, the appropriate protected amino acid moiety (e.g., compound A-1 in Scheme A) in N-methyl morpholine is coupled to *p*-nitraniline (Aldrich Chemical Co.) at about 0° C in the presence of isobutyl chloroformate in an aprotic solvent (e.g., THF) under an inert atmosphere by stirring for 12-24 hr, preferably 12 hr. The product is purified by chromatography and deprotected by hydrolysis in 4 N HCl in dioxane. Following their preparation, the P_1 moieties are coupled (e.g., via EDC methods described herein and in the reaction schemes) with the

-73-

appropriate P_2P_3 moiety (e.g., compound A-4 in Reaction Scheme A), or with the appropriate P_2 moiety (e.g., compound J-5 in Reaction Scheme J) to produce the chromogenic substrates of formula (I) and (II).

Fluorogenic substrates wherein X is 7-amino-4-methylcoumarin (AMC) may be prepared, for example, as follows. The appropriate protected amino acid moiety (e.g., compound A-1 in Reaction Scheme A) in N-methyl morpholine is coupled to 7-amino-6-methylcoumarin (Aldrich Chemical Co.) at about -15°C in the presence of iso-butyl chloroformate in an aprotic solvent (e.g., THF) under an inert atmosphere by stirring for 12-24 hr, preferably 12 hr. The product is purified by chromatography and deprotected by hydrolysis in 4 N HCl in dioxane. Following their preparation, the P_1 moieties are coupled (e.g. via EDC methods described herein and in the reaction schemes) with the appropriate P_2P_3 moiety (e.g., compound A-4 in Reaction Scheme A), or with the appropriate P_2 moiety (e.g., compound J-5 in Reaction Scheme J) to produce the fluorogenic substrates of formula (I) and (II).

In addition, the resulting compounds may be deprotected by hydrolysis in 4 N HCl in dioxane and coupled with succinic acid, decanoic acid or 6-amino caproic acid thereby generating a free carboxylate or amino group at the amino terminus for coupling the compounds to a solid resin support (e.g., cyanogen bromide-conjugated Sepharose) for identification and isolation of proteases.

To use the fluorogenic and chromogenic substrates in methods of identifying protease inhibitors, the substrates are incubated, in the presence and absence of test compound, with a selected purified protease (or a cell lysate fraction enriched for the protease activity) in a suitable buffer for optimum protease activity (e.g., 100 μl of 0.1 M potassium phosphate buffer, pH 7.5, supplemented with 5 mM DTT and 5 mM EDTA). Cleavage of the substrate by the protease liberates the fluorophore or chromophore. Test compounds that inhibit this cleavage are those of interest.

-74-

The liberated chromophores or fluorophores can be readily detected. For example, fluorescence of the freed fluorophore may be detected using standard fluorometry by applying excitation radiation of the appropriate wavelength for the particular fluorophore used (e.g., 360 nm for AMC) followed by measurement of the resulting emitted radiation of a different wavelength (e.g., 460 nm for AMC). [For further details of fluorescence detection of fluorophores see Kirschke and Wiederanders (1995) Meth. Enzymol. 244:500-511.] Likewise, color emitted by a released chromophore may be detected using a spectrophotometer and standard procedures (e.g., detection of free nitranilide at 405 nm).

Test compounds that inhibit fluorescence or color emission resulting from cleavage of the substrate by proteases may be potential inhibitors of the protease. The protease inhibitor identification methods using the chromogenic and fluorogenic substrates provide rapid, cell-free assays amenable to high through-put procedures for screening large numbers of test compounds and identifying compounds that are candidate inhibitors.

Compounds that are positive in the protease inhibitor assay may be further characterized using any of the assay systems described herein. For example, when a compound is positive in an assay employing a fraction enriched for APP-processing activity, it may be further evaluated in assays described as in Example 31 for identifying compounds having activity as modulators of APP processing.

The following specific examples further illustrate the methods by which compounds of formulae I and II may be prepared but are not meant to limit the scope of this invention to the specific compounds. Thus, the following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

-75-

EXAMPLE 1

Preparation of *N*-L-(methyl)Nle *N*-methoxy-*N*-methylamide hydrochloride

To a stirred solution of *N*-Boc-L-Nle-OH (5.0 g, 22.0 mmol) in anhydrous methylene chloride (CH_2Cl_2) (25 mL) under Argon (Ar) at room temperature (R.T.) were added successively hydroxybenzotriazole hydrate (HOBT) (5.8 g, 43.0 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (4.1g, 22.0 mmol) *N,O*-dimethylhydroxylamine hydrochloride (1.9 g, 20.0 mmol) and triethylamine (Et_3N) (3 mL, 22.0 mmol). The reaction mixture was stirred for 16 h. Additional CH_2Cl_2 (25 mL) was added and the mixture was washed with saturated aqueous sodium hydrogen carbonate (sat. NaHCO_3) (2 x 10 mL), 10% aqueous hydrogen chloride (10% HCl) (2 x 10 mL), saturated aqueous sodium chloride (sat. NaCl) (2 x 10 mL), dried over anhydrous magnesium sulfate (MgSO_4), filtered and concentrated. The resultant residue was purified by flash chromatography on silica gel (ethyl acetate : hexane (EA:H); 1:3) to give *N*-Boc-L-Nle *N*-methoxy-*N*-methylamide as a colorless oil (4.1 g, 69.3%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.87 - 0.92 (m, 3 H), 1.24 - 1.72 (m, 15 H), 3.21 (s, 3 H), 3.78 (s, 3H), 4.66 - 4.68 (m, 1 H), 5.16 (d, 1 H, $J = 9.0$ Hz) ppm.

To a stirred solution of the *N*-Boc-L-Nle *N*-methoxy-*N*-methylamide (1.59 g, 5.5 mmol) in 20:1 anhydrous tetrahydrofuran: dimethylformamide (THF:DMF) (22 mL) at 0°C under Ar was added methyl iodide (0.68 mL, 11.0 mmol) and 60% sodium hydride (NaH) in oil dispersion (0.25 g, 6.0 mmol). The reaction mixture was refluxed for 16 h. The mixture was poured onto 10% HCl and was extracted with EA (3 x 20 mL). The combined organic extracts were washed with sat. NaCl (2 x 10 mL), dried (MgSO_4), filtered and concentrated *in vacuo* to afford *N*-Boc-L-(methyl)Nle *N*-methoxy-*N*-methylamide derivative as a yellow oil (0.86 g, 54.5%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.87 - 0.93 (m, 3 H), 1.25 - 1.48 (m, 13 H), 1.71 - 1.73 (m, 2 H), 2.83 - 2.85 (m, 3 H), 3.19 (s, 3 H), 3.69 - 3.74 (d, 3 H, $J = 15.0$ Hz), 4.80-4.95 (broad (b)-m, 1 H) ppm.

-76-

N-Boc- L-(methyl)Nle *N*-methoxy-*N*-methylamide (0.84 g, 2.9 mmol) was treated with 4 N HCl in dioxane (15 mL) at R.T. The reaction mixture was stirred for 1.5 h then concentrated *in vacuo*. The solid was treated with anhydrous ether (3 x 10 mL) and concentrated *in vacuo*. The resulting

5 *N*-L-(methyl)Nle *N*-methoxy-*N*-methylamide hydrochloride was isolated as a white solid (0.47 g, 85.7%): ¹H NMR (CDCl₃, 300 MHz) δ 0.91 - 0.95 (m, 3 H), 1.73 (m, 4 H), 2.06 - 2.08 (m, 2 H), 2.27 - 2.29 (m, 6 H), 3.71 - 4.09 (m, 3 H) ppm.

EXAMPLE 2

10 Preparation of *N*-Cbz-L-Leu-L-Leu-DL-(methyl)norleucinal

To a stirred solution of *N*-L-(methyl)Nle *N*-methoxy-*N*-methylamide hydrochloride isolated above in Example 1 (0.87 g, 2.3 mmol) in anhydrous CH₂Cl₂ (20 mL) under Ar at R.T. were added HOBT (0.62 g, 4.6 mmol), EDC (0.44 g, 2.3 mmol), *N*-Cbz-L-Leu-L-Leu-OH (0.47 g, 2.1 mmol) and Et₃N (0.3

15 mL, 2.3 mmol). The reaction mixture was stirred for 16 h then taken up in additional CH₂Cl₂ (20 mL). The mixture was washed with sat. NaHCO₃ (2 x 10 mL), 10% HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated. The resulting crude was purified by flash chromatography on silica gel (EA:H, 1:3) to yield *N*-Cbz-L-Leu-L-Leu- L-

20 (methyl)Nle *N*-methoxy-*N*-methylamide as a colorless oil (0.4 g, 34.9%): ¹H NMR (CDCl₃, 300 MHz) δ 0.87 - 1.01 (m, 3 H), 1.16 - 1.95 (m, 12 H), 2.95 - 3.21 (m, 6 H), 3.65 - 3.82 (m, 3 H), 4.23 (m, 1 H), 4.97 - 5.52 (m, 5 H), 6.72 - 6.75 (m, 1 H), 7.30 - 7.36 (m, 5 H) ppm.

To a stirred solution of *N*-Cbz-L-Leu-L-Leu-L-(methyl)Nle *N*-methoxy-*N*-methylamide (0.33 g, 0.55 mmol) in anhydrous THF (3 mL) under Ar at

25 -78° C was added a solution of 1.0 M diisobutylaluminum hydride (DIBAL) in hexanes (2.7 mL, 2.7 mmol). The reaction mixture was stirred for 1 h at -78° C then quenched by adding a saturated aqueous Rochelle salt solution (15 mL). The mixture was extracted with EA (3 x 20 mL). The combined

30 organic extracts were dried (MgSO₄), filtered, concentrated and purified on

-77-

silica gel (EA:H; 2:3) to afford the title compound *N*-Cbz-L-Leu-L-Leu-DL-(methyl)norleucinal as a colorless oil (0.12 g, 45%): Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.93 - 1.01 (overlapping (ol)-m, 15 H), 1.25 - 1.71 (ol-m, 12 H), 4.24 (ol-m, 1 H), 4.52 (ol-m, 1 H), 5.03 - 5.13 (ol-m, 3 H), 7.32 - 7.36 (ol-m, 5 H), 9.50 - 9.56 (ol-m, 1 H) ppm.

EXAMPLE 3**Preparation of *N*-Ac-L-Leu-L-Leu-DL-cyclohexylalaninal**

To a stirred solution of *N*-Ac-L-Leu-OH (5.0 g, 28.9 mmol) in anhydrous CH₂Cl₂ (50 mL) at R.T. under Ar were added HOBT (7.9 g, 57.7 mmol), EDC (5.5 g, 28.9 mmol), H-L-Leu-OBn *p*-TsOH (9.9 g, 24.9 mmol) and Et₃N (4 mL, 28.9 mmol). The reaction mixture was stirred for 16 h then taken up in addition CH₂Cl₂ (20 mL). The organic was washed with sat. NaHCO₃ (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated to afford *N*-Ac-L-Leu-L-Leu-OBn as an oil (9.0 g, 96%): ¹H NMR (CDCl₃, 300 MHz) δ 0.87 - 0.90 (m, 12 H), 1.43 - 1.68 (m, 6 H), 1.94, +1.96 (s + s, 3 H), 4.51 - 4.63 (m, 2 H), 5.09 - 5.19 (m, 2 H), 6.61 - 6.64 (d, 1 H, *J* = 9.0 Hz), 6.95 - 6.97 (d, 1 H, *J* = 6.0 Hz), 7.28 - 7.39 (m, 5 H) ppm.

To a stirred solution of *N*-Ac-L-Leu-L-Leu-OBn (9.09 g, 23.9 mmol) in EA (100 mL) at R.T. was added 20% palladium hydroxide on carbon (Pd(OH)₂/C) (0.9 g, 10% by weight). The reaction mixture was placed under 20 psi hydrogen (H₂). After 1 h the reaction mixture was filtered through celite and concentrated *in vacuo* to give *N*-Ac-L-Leu-L-Leu-OH as a white solid (6.0 g, 87.67%): ¹H NMR (300 MHz, CDCl₃) δ 0.82 - 0.90 (m, 12 H), 1.39 - 1.82 (m, 9 H), 4.18 - 4.34 (m, 2 H), 7.94 - 8.13 (m, 2 H) ppm.

To a stirred solution of *N*-Ac-L-Leu-L-Leu-OH (1.0 g, 3.5 mmol) in anhydrous CH₂Cl₂ (10 mL) at R.T. under Ar was added 1,1-carbonyldiimidazole (CDI) (0.68 g, 4.2 mmol). Then Et₃N (1.0 mL, 7.0 mmol) and cyclohexylalanine hydrochloride (0.73 g, 3.5 mmol) were successively added. After 16 h of stirring the reaction mixture was

-78-

concentrated. The residue was triturated with 1 N aqueous hydrogen chloride (1 N HCl), washed with water and dried *in vacuo* to afford the *N*-Ac-L-Leu-L-Leu-L-Ala(cyclohexyl)-OH acid as a white solid (0.66 g, 42.5%): ¹H NMR (CDCl₃, 300 MHz) δ 0.90 - 0.98 (m, 12 H), 1.15 - 1.82 (m, 19 H),
5 1.95 - 1.98 (m, 3 H), 4.36 - 4.46 (m, 3 H) ppm.

To a stirred solution of *N*-Ac-L-Leu-L-Leu-L-Ala(cyclohexyl)-OH (0.6 g, 1.2 mmol) in anhydrous DMF (10 mL) at R.T. under Ar were added HOBT (0.26 g, 1.3 mmol), 4-dimethylaminopyridine (DMAP) (0.05 g) and 1-thiol propane (0.12 mL, 1.3 mmol). The reaction mixture was stirred for 16 h,
10 then poured onto EA/10% HCl (30mL/10mL). The organic layer was separated and washed with sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated. Purification by flash chromatography on silica gel (EA:H; 1:3) afforded the *N*-Ac-L-Leu-L-Leu-L-Ala(cyclohexyl)-SCH₂CH₂CH₃ as a white solid (0.3 g, 44.4%): ¹H NMR (CDCl₃, 300 MHz) δ 0.86 - 0.98 (m, 15 H),
15 1.49 - 2.07 (m, 24 H), 2.76 - 2.87 (m, 2 H), 4.64 - 4.80 (m, 3 H) ppm.

To a solution of the thiol ester (0.27 g, 0.52 mmol) in anhydrous CH₂Cl₂ at R.T. under Ar were added 10% palladium on carbon (Pd/C) (27 mg, 10% by weight) and triethylsilane (Et₃SiH) (0.43 mL, 2.6 mmol). The mixture was stirred for 16 h, then filtered through celite and concentrated.
20 The residue was purified by flash chromatography on silica gel (EA:H; 1:3→ EA) to afford the title compound *N*-Ac-L-Leu-L-Leu-DL-cyclohexylalanine as a white solid (0.12 g, 54.79%): Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.85 - 1.98 (ol-m, 31 H), 1.97 + 1.98 (s + s, 3 H), 4.25 - 4.38 (ol-m, 2 H), 4.60-4.78 (ol-m, 1 H), 7.00-8.00 (ol-m, 3 H),
25 9.48 + 9.54 (s + s, 1 H) ppm.

EXAMPLE 4

Preparation of (2S)-*N*-Ac-L-Leu-L-Leu *N*-[2-(hexanenitrile)]amide

To a stirred solution of *N*-Boc-L-Nle-OH (0.5 g, 2.2 mmol) in anhydrous THF (15 mL) under Ar at -23° C was added 4-methylmorpholine
30 followed by *iso*-butyl chloroformate (0.21 mL, 2.2 mmol). The reaction

-79-

mixture was then treated with gaseous NH_3 for 1 h. Stirring was continued at -23°C for an additional 2 h. The mixture was warmed to R.T. and then poured onto 10% aqueous citric acid (20 mL). The mixture was extracted with EA (3 x 50 mL). The combined organics were washed with sat.

5 NaHCO_3 (3 x 25 mL), sat. NaCl (2 x 25 mL), dried (MgSO_4), filtered and concentrated to afford *N*-Boc-L-Nle- NH_2 as a white solid (0.48 g, 91%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.84 - 0.92 (m, 3 H), 1.22 - 1.31 (m, 4 H), 1.41 (s, 9 H), 1.49 - 1.59 (m, 1 H), 1.74 - 2.01 (m, 1 H), 4.03 - 4.1 (m, 1 H), 5.06 (d, 1 H), 5.75 (s, 1 H), 6.25 (s, 1 H) ppm.

10 To a stirred solution of the amide (*N*-Boc-L-Nle- NH_2) (0.16 g, 0.67 mmol) in anhydrous CH_2Cl_2 (4 mL) at R.T. under Ar were added anhydrous pyridine (0.27 mL, 3.4 mmol) and *p*-toluenesulfonyl chloride (0.26 g, 1.34 mmol). The reaction mixture was stirred for 3 days, then treated with sat. NaHCO_3 (4 mL). The mixture was stirred for 1 h. The organic was
15 separated and the aqueous was extracted with CH_2Cl_2 (2 x 10 mL). The combined organics were washed with 1 N HCl (2 x 5 mL), dried (MgSO_4), filtered and concentrated to give a crude residue. Purification by flash chromatography on silica gel (EA) afforded (2*S*)-*N*-Boc-2-amino-hexanenitrile as an oil (0.14 g, 100%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.93 (t, 3 H, J =
20 7.3 Hz), 1.25 - 1.52 (m, 13 H), 1.75 - 1.91 (m, 2 H), 4.50 - 4.61 (m, 1 H), 4.82 (bs, 1 H) ppm.

To the nitrile (1.0 g, 4.71 mmol) was added 4 N HCl/dioxane (10 mL). The reaction mixture was stirred at R.T. for 30 min. The resultant precipitate was filtered and washed with hexanes (3 x 5 mL) to afford (2*S*)-
25 2-amino-hexanenitrile hydrochloride as a white solid (0.68 g, 97.59%): ^1H NMR (CD_3OD , 300 MHz) δ 0.95-0.99 (m, 3 H), 1.10-1.60 (m, 4 H), 1.89-2.00 (m, 2 H), 4.20-4.30 (m, 1 H) ppm.

To a stirred solution of *N*-Ac-L-Leu-L-Leu-OH (0.87 g, 3.0 mmol) in anhydrous CH_2Cl_2 (50 mL) at R.T. under Ar was added EDC (0.58 g, 3.0
30 mmol), HOBT (0.81 g, 6.0 mmol) and Et_3N (0.41 mL). After 16 h, the

-80-

reaction mixture was washed with sat. NaHCO_3 (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO_4), filtered and concentrated. Purification by flash chromatography on silica gel (EA) gave the title compound as a white solid (0.42 g, 35%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.82 - 0.92 (m, 15 H), 1.21 - 1.99 (m, 12 H), 2.02 (s, 3 H), 4.51 (m, 1 H), 4.64 (m, 1 H), 6.78 (m, 1 H), 7.50 (m, 1 H), 8.06 (m, 1 H) ppm.

EXAMPLE 5

Preparation of *N*-Cbz-L-Leu-L-Leu-DL-Nle- CO_2Et

To a stirred solution of *N*-Cbz-L-Leu-L-Leu-L-Nle-OH (1.0 eq) in anhydrous THF at R.T. under Ar is added DMAP (cat.), pyridine (1.0 eq), ethyl oxalyl chloride (2.0 eq). The mixture is gently refluxed for 3 h. The mixture is treated with water, stirred for 30 min. at R.T. and then extracted with EA. The organic extracts are dried (MgSO_4), filtered and concentrated to afford enol ester product as a crude residue. To a stirred suspension of the crude residue (1.0 eq) in anhydrous ethanol at R.T. under Ar is added dropwise a solution of sodium ethoxide in anhydrous ethanol. The reaction mixture is stirred for 3 h. The ethanol is then removed and the residue is treated with ether. The ether solution is washed with water, dried (MgSO_4) and evaporated to give a crude residue. This residue is purified by flash chromatography on silica gel to give the peptide ketoester *N*-Cbz-L-Leu-L-Leu-DL-Nle- CO_2Et .

EXAMPLE 6

Preparation of *N*-Cbz-L-Leu-L-Leu-DL-Nle- CO_2H

To a stirred solution of *N*-Cbz-L-Leu-L-Leu-DL-Nle- CO_2Et (1.0 eq), as prepared in Example 5, in methanol at R.T. is added 1 N NaOH (1.1 eq). The mixture is stirred for 6 h. The reaction mixture is cooled to 0°C , acidified with 1 N HCl (pH = 3) and extracted with EA. The organic extracts are washed with water, dried (MgSO_4), filtered and concentrated to give a crude residue. Trituration with hexane and drying *in vacuo* gives the title compound, *N*-Cbz-L-L u-L-Leu-DL-Nle- CO_2H .

-81-

EXAMPLE 7

Preparation of *N*-Cbz-L-Leu-L-Leu-DL-Nle-CONHEt

To a stirred solution of *N*-Cbz-L-Leu-L-Leu-DL-Nle-CO₂Et (1.0 eq), as prepared in Example 5, in anhydrous CH₂Cl₂ at R.T. is added 1,2-ethanedithiol (2.2 eq), followed by boron trifluoride etherate (0.1 eq). The mixture is stirred for 16 h. Water and ethyl ether are added. The organic layer is separated, washed with water, sat. NaCl, dried (MgSO₄), filtered and concentrated to afford the protected α -ketoester.

The protected α -ketoester is dissolved in ethanol (5 mL) and cooled to 0° C, and ethylamine is bubbled through the solution. The mixture is warmed to R.T., stirred overnight, filtered and concentrated. The crude residue is purified by flash chromatography on silica gel to give the title compound *N*-Cbz-L-Leu-L-Leu-DL-Nle-CONHEt.

EXAMPLE 8

15 Preparation of the precursor ethyl 2-amino-4-methyl-4-pentenoate hydrochloride

To a solution of *N*-(diphenylmethylene)glycine ethyl ester (6.6 g, 24.7 mmol) in anhydrous THF at -78° C under Ar was slowly added 1.0 M lithium bis(trimethylsilyl)amide (LiHMDSi) in THF (24.7 mL, 24.7 mmol) over 15 min. Stirring was continued for 30 min at -78° C, then 3-bromo-2-methylpropene (2.5 mL, 25.0 mmol) was added. The mixture was gradually warmed to R.T. then stirred for 1 h at R.T. The reaction mixture was treated with water and then concentrated. The residue was taken up in EA (50 mL). The organic layer was washed with sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated. The crude was purified by flash chromatography on silica gel (EA:H₂O: 1:4) to give ethyl 4-methyl-2-[(diphenylmethylene)amine]-4-pentenoate as a colorless oil (6.4 g, 89.3%): ¹H NMR (CDCl₃, 300 MHz) δ 1.23 - 1.29 (t, 3 H, *J* = 6.0 Hz), 1.48 - 1.49 (m, 3 H), 2.55 - 2.69 (m, 2 H), 4.11 - 4.24 (m, 3 H), 4.71 - 4.75 (m, 2 H), 7.16 - 7.83 (m, 10 H) ppm.

-82-

To a stirred solution of the above ethyl ester (6.4 g, 20.0 mmol) in anhydrous ether (15 mL) at R.T. was added 1 N HCl (70 mL). After 40 min, the two phases were separated, and the aqueous layer was washed with ether (3 x 10 mL). The aqueous layer was adjusted with 1 N NaOH (pH = 10), then extracted with ether (3 x 20 mL). The combined organic extracts were dried (MgSO₄), filtered and then adjusted with 4 N HCl/dioxane (pH = 3) and concentrated *in vacuo* to afford ethyl 2-amino-4-methyl-4-pentenoate hydrochloride as an oil (3.11 g, 79.4%): ¹H NMR (CDCl₃, 300 MHz) δ 1.25 - 1.32 (t, 3 H, *J* = 6.0 Hz), 1.81 (s, 3 H), 2.74 - 2.81 (m, 2 H), 4.22 - 4.29 (m, 2 H), 4.98 (d, 2 H, *J* = 12.0 Hz) ppm.

EXAMPLE 9

Preparation of (2*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[2-(4-methyl-4-pentenal)]amide

To a stirred solution of *N*-Cbz-L-Leu-OH (5.6 g, 20.7 mmol) in anhydrous CH₂Cl₂ (50 mL) at R.T. under Ar were added successively HOBT (5.6 g, 41.5 mmol), EDC (4.0 g, 20.7 mmol), H-L-Leu-OCH₃ HCl (3.4 g, 18.8 mmol) and Et₃N (2.9 mL, 20.7 mmol). The reaction mixture was stirred for 16 h. The mixture was taken up in additional CH₂Cl₂ (30 mL), washed with sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated. The resulting residue was purified by flash chromatography on silica gel (EA:H; 1:2) to afford the dipeptide *N*-Cbz-L-Leu-L-Leu-OCH₃ as a white solid (6.0 g, 64.1%): ¹H NMR (CDCl₃, 300 MHz) δ 0.86 - 0.95 (ol-t, 12 H, *J* = 6.0 Hz), 1.48 - 1.74 (m, 6 H), 3.73 (s, 3 H), 4.20 - 4.25 (m, 1 H), 4.56 - 4.63 (m, 1 H), 5.11 (s, 2 H), 5.20 (d, 1 H, *J* = 6.0 Hz), 6.36 (d, 1 H, *J* = 6.0 Hz), 7.31 - 7.39 (m, 5 H) ppm.

To a stirred solution of *N*-Cbz-L-Leu-L-Leu-OCH₃ (6.0 g, 14.6 mmol) in MeOH/H₂O (3:1) (60 mL) at R. T. was added lithium hydroxide monohydrate (LiOH.H₂O) (1.0 g, 43.9 mmol) and hydrogen peroxide H₂O₂ (30% weight in H₂O) (4.5 mL, 43.9 mmol). The reaction mixture was stirred for 3.5 h and then quenched with 10% HCl. The resulting mixture was extracted with EA (3 x 20 mL). The combined organic extracts were

-83-

washed with sat. NaCl (2 x 10 mL), dried (MgSO₄) and concentrated *in vacuo* to afford *N*-Cbz-L-Leu-L-Leu-OH as a white solid (5.0 g, 90.5%): ¹H NMR (CDCl₃, 300 MHz) δ 0.89 - 0.92 (t, 12 H, *J* = 3.0 Hz), 1.48 - 1.71 (m, 6 H), 4.11 - 4.13 (m, 1 H), 4.55 - 4.62 (m, 1 H), 5.09 (s, 2 H), 5.64 (d, 1 H, *J* = 9.0 Hz), 6.83 (d, 1 H, *J* = 9.0 Hz), 7.33- 7.40 (m, 5 H) ppm.

To a stirred solution of *N*-Cbz-L-Leu-L-Leu-OH (3.2 g, 8.5 mmol) in anhydrous CH₂Cl₂ (35 mL) at R. T. under Ar were added HOBT (2.3 g, 17.1 mmol), EDC (1.6 g, 8.5 mmol), ethyl 2-amino-4-methyl-4-pentenoate hydrochloride, as prepared in Example 8, (1.5 g, 7.8 mmol) and Et₃N (1.2 mL, 8.5 mmol). The reaction mixture was stirred for 16 h at R.T. The mixture was taken up in CH₂Cl₂ (30 mL). The organic layer was washed with sat. NaHCO₃ (2 x 10 mL), 10% HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated. The residue was purified by flash chromatography on silica gel (EA:H; 1:4) to afford (2*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[2- [ethyl (4-methyl-4-pentenoate)]amide as a white solid (1.9 g, 47.3%): Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.88 - 0.92 (ol-m, 12 H), 1.21 - 1.29 (ol-m, 3 H), 1.51 - 1.71 (ol-m, 9 H), 2.39 - 2.52 (ol-m, 2 H), 4.13 - 4.21 (ol-m, 3 H), 4.47 - 4.82 (ol-m, 4 H), 5.10 (ol-m, 2 H), 5.32 (d, 1 H, *J* = 9.0 Hz), 6.44 - 6.77 (m, 2 H), 7.29 - 7.36 (ol-m, 5 H) ppm.

To a stirred solution of the above ethyl ester (1.9 g, 3.6 mmol) in anhydrous THF (10 mL) at 0° C under Ar was added lithium borohydride (LiBH₄) (0.16 g, 7.1 mmol). Stirring was continued for 30 min at 0° C then the mixture was warmed to R. T. After 1 h, 1 N HCl (1 mL) was added to the reaction mixture, and then extracted with EA (3 x 20 mL). The combined organic were washed with sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated *in vacuo* to afford the crude residue. The residue was purified by flash chromatography on silica gel (EA:H; 1:3) to yield the alcohol as a white solid (1.4 g, 85.6%): Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.88 - 0.95 (ol-m, 12 H), 1.47 -

-84-

1.73 (ol-m, 9 H), 2.16 - 2.27 (ol-m, 2 H), 3.54 - 3.67 (ol-m, 2 H), 4.12 (ol-m, 2 H), 4.33 - 4.40 (ol-m, 1 H), 4.74 - 4.80 (ol-m, 2 H), 5.03 - 5.37 (ol-m, 3 H), 6.34 - 6.37 (ol-m, 2 H), 7.30 - 7.40 (ol-m, 5 H) ppm.

To a stirred solution of anhydrous dimethyl sulfoxide (DMSO) (0.18 mL, 2.25 mmol) in anhydrous THF (5 mL) at -78° C under Ar was added oxalyl chloride (0.24 mL, 2.25 mmol) dropwise over 5 min. After 30 min, the reaction mixture was treated with a solution of the alcohol (0.5 g, 1.0 mmol) in anhydrous THF (5 mL). Stirring was continued at -78° C for an additional 30 min, then Et₃N (0.65 mL, 4.7 mmol) was added. The reaction mixture was warmed to 0° C and stirred for 15 min. EA (50 mL) was added to the mixture and the organic layer was washed with sat. NaHCO₃ (2 x 10 mL), 10% HCl (2 x 10 mL) and sat. NaCl (2 x 10 mL). The organic layer was dried (MgSO₄), filtered and concentrated to afford a crude residue. Purification of the crude by flash chromatography on silica gel (kieselgel 60 silanisiert) (EA:H; 1:5) yielded the title compound as a white solid (0.23 g, 47.8%): Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.88 - 0.98 (ol-m, 12 H), 1.52 - 1.73 (ol-m, 9 H), 2.29 - 2.58 (ol-m, 2 H), 4.19 (ol-m, 1 H), 4.49-4.51 (ol-m, 2 H), 4.76 - 4.86 (ol-m, 2 H), 5.00 - 5.09 (ol-m, 2 H), 5.39 - 5.42 (ol-m, 1 H), 6.70 - 7.14 (ol-m, 2 H), 7.34 - 7.42 (ol-m, 5 H) ppm.

EXAMPLE 10

Preparation of (2S)-N-Cbz-L-Leu-L-Leu N-[2-(thiazole-oxo-pentyl)]amide

To a stirred solution of thiazole (0.11 mL, 1.55 mmol) in anhydrous ether (8 mL) at -78° C under Ar was slowly added 1.8 M N-butyllithium in hexanes (*n*BuLi) (1.0 mL, 1.7 mmol). After an addition 20 min of stirring at -78° C N-Boc-L-Nle N-methoxy-N-methylamide (0.17 g, 0.62 mmol) in anhydrous ether (5 mL) was added. Stirring was continued for 1 h at -78° C then gradually warmed to R. T. The resulting mixture was treated with 1 N HCl (1 mL), 1 N NaOH (pH = 9), and extracted with ether (3 x 10 mL). The combined organic layers were washed with sat. NaHCO₃ (2 x 10 mL).

-85-

sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated. Purification by flash chromatography on silica gel (EA:H; 1:5) afforded the (2S)-N-Boc-2-amino-thiazole-oxo-pentyl derivative as a white solid (0.14 g, 76%): ¹H NMR (CDCl₃, 300 MHz) δ 0.86 (t, 3 H, J = 6.0 Hz), 1.23 - 1.62 (m, 13 H), 1.66 - 1.75 (m, 2 H), 5.32 - 5.46 (m, 2 H), 7.70 (d, 1 H, J = 3.0 Hz), 8.03 (d, 1 H, J = 6.0 Hz) ppm.

The above derivative (0.13 g, 0.44 mmol) was treated with 4 N HCl/dioxane (5 mL) at R. T. After 30 min the reaction mixture was concentrated *in vacuo*. The resulting solid was recrystallized (MeOH/Ether) to give the hydrochloride as a white solid (0.1 gr, 75%): ¹H NMR (CD₃OD, 300 MHz) δ 0.64 - 0.73 (t, 3 H, J = 6.0 Hz), 1.17 - 1.21 (m, 4 H), 1.76 - 1.99 (m, 2 H), 4.86 - 4.91 (m, 1 H), 7.72 - 7.98 (m, 2 H) ppm.

To the resulting hydrochloride (0.09 g, 0.33 mmol) in anhydrous CH₂Cl₂ (10 mL) at R. T. under Ar was added HOBT (0.1 g, 0.73 mmol), EDC (0.07 g, 0.36 mmol), N-Cbz-L-Leu-L-Leu-OH (0.09 g, 0.33 mmol) and Et₃N (0.09 mL, 0.66 mmol). After 16 h CH₂Cl₂ (20 mL) was added, and the organic layer was washed with sat. NaHCO₃ (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated. Purification by flash chromatography on silica gel (EA:H;1:1) afforded the title compound as a white solid (0.16 gr, 88%): ¹H NMR (CDCl₃, 300 MHz) δ 0.83 - 1.06 (m, 15 H), 1.20 - 2.15 (m, 12 H), 4.10 - 4.30 (m, 1 H), 4.48 - 4.61 (m, 1 H), 5.11 (s, 2 H), 5.15 - 5.25 (m, 1 H), 5.60 - 5.70 (m, 1 H), 6.32 - 6.50 (m, 1 H), 7.30 - 7.40 (m, 5 H), 7.70 (dd, 1 H, J = 6.0, 3.0 Hz), 8.05 (dd, 1 H, J = 6.0, 3.0 Hz) ppm.

25

EXAMPLE 11

Preparation of N-Ac-L-Leu-L-Leu-L-Nle-COCHN₂

To a stirred solution of N-Ac-L-Leu-L-Leu-L-Nle-OH (0.3 g, 0.77 mmol), in anhydrous THF (10 mL) at -23° C were added 4-methylmorpholine (0.1 mL, 0.85 mmol) and *iso*-butyl chloroformate (0.11 mL, 0.85 mmol). The mixture was stirred at -23° C for 20 min. The resulting mixture was

30

-86-

added to a solution of diazomethane (10.0 mmol) in anhydrous ether (5 mL) at 0° C. The mixture was stirred at 0° C for an additional 1 h then gradually warmed to R. T., and stirred for an addition 3 h at R. T. The reaction mixture was taken up in CH₂Cl₂ (30 mL) and the organic layer was washed
5 with sat. NaHCO₃ (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO₄) and concentrated. Recrystallization (EA/H) afforded the desired diazomethyl ketone (N-Ac-L-Leu-L-Leu-L-Nle-COCHN₂) as a white solid (0.33 g, 92%):
1H NMR (CDCl₃, 300 MHz) δ 0.88 - 0.99 (m, 15 H), 1.23 - 1.90 (m, 12 H), 2.00 (s, 3 H), 4.53 - 4.68 (m, 1 H), 4.72 - 4.79 (m, 2 H), 5.86 (s, 1 H),
10 7.71 (broad doublet (bd) 1 H), 7.96 (bd, 1 H), 8.16 (bd, 1 H) ppm.

EXAMPLE 12

Preparation of (3*SR*)-*N*-Ac-L-Leu-L-Leu *N*-[3-(1,1,1-trifluoro-2-oxo-heptyl)]amide

To a stirred solution of 1-nitropentane (1.0 g, 8.5 mmol) and
15 trifluoroacetaldehyde ethyl hemiacetal (1.2 mL, 8.5 mL) was added potassium carbonate (K₂CO₃) (0.06 g, 0.43 mmol). The reaction mixture was heated at 60° C under Ar for 3 h. The mixture was cooled to R.T., then taken up in EA (50 mL). The organic layer was washed with 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated to
20 give (2*SR*)-(3*SR*)-3-nitro-1,1,1-trifluoro-2-heptanol as a crude oil (1.7 g, 93%): 1H NMR (CDCl₃, 300 MHz) δ 0.91 (t, 3 H, *J* = 7.2 Hz), 1.24 - 1.43 (m, 4 H), 2.04 - 2.11 (m, 2 H), 4.08 - 4.77 (m, 3 H) ppm.

To a stirred solution of the nitro-alcohol derivative (1.6 g, 7.4 mmol) in methanol (10 mL) was added Raney Nickel (0.16 g, 10% by weight). The
25 mixture was placed under 35 psi of H₂ for 16 h, and then was filtered through celite. The celite was washed with methanol (3 x 10 mL). The combined organics were concentrated to give (2*SR*)-(3*SR*)-3-amino-1,1,1-trifluoro-2-heptanol as an oil (0.8 g, 64.9%): 1H NMR (CD₃OD, 300 MHz) δ 0.89 - 0.95 (m, 3 H), 1.20 - 2.10 (m, 6 H), 3.30 - 4.40 (m, 4 H) ppm.

-87-

To a stirred solution of (2*SR*)-(3*SR*)-3-amino-1,1,1-trifluoro-2-heptanol (0.36 g, 2.14 mmol) in anhydrous CH_2Cl_2 (20 mL) were added *N*-Ac-Leu-Leu-OH (0.67 g, 2.4 mmol), HOBT (0.33 g, 2.4 mmol), EDC (0.46 g, 2.4 mmol) and Et_3N (0.33 mL, 2.4 mmol). The reaction mixture was stirred for 18 h then washed with sat. NaHCO_3 (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO_4), filtered and concentrated. Purification by flash chromatography on silica gel (EA:H; 1:2) afforded the trifluoromethyl alcohol peptide derivative as a white solid (0.85 g, 88.8%): Reporting a mixture of diastereomers ^1H NMR ($\text{DMSO}-d_6$, 300 MHz) δ 0.89 - 0.95 (ol-m, 15 H), 1.05 - 1.90 (ol-m, 12 H), 1.95 (s, 3 H), 3.91 - 4.50 (ol-m, 4 H) ppm.

To a stirred solution of the above product (0.20 g, 0.44 mmol) at R. T. under Ar (0.2 g, 0.44 mmol) in anhydrous 1:1 THF/ CH_2Cl_2 (40 mL) were added trifluoroacetic acid (TFA) (0.10 mL, 1.32 mmol) and the Dess-Martin reagent (0.56 g, 1.32 mmol). The reaction mixture was stirred for 24 h then concentrated *in vacuo*. The residue was treated with a mixture of EA (20 mL), sat. NaHCO_3 (5 mL) and saturated aqueous sodium thiosulfate (sat. $\text{Na}_2\text{S}_2\text{O}_3$) (5 mL). The organic layer was separated and washed with a sat. NaHCO_3 (2 x 5 mL), sat. $\text{Na}_2\text{S}_2\text{O}_3$ (2 x 5 mL), sat. NaCl (2 x 5 mL), dried (MgSO_4), filtered and concentrated. The residue was recrystallized (EA/H) to give the title compound as a white solid (0.11 g, 55%): Reporting a mixture of diastereomers ^1H NMR ($\text{DMSO}-d_6$, 300 MHz) δ 0.81 - 0.87 (ol-m, 15 H), 1.09 - 1.56 (ol-m, 12 H), 1.98 (ol-s, 3 H), 3.90 - 4.0 (ol-m, 1 H), 4.20 - 4.32 (ol-m, 2 H) ppm.

EXAMPLE 13

Preparation of (2*SR*)-H-L-Leu *N*-[2-(ethyl 4-methyl-4-pentenoate)]amide hydrochloride

To a stirred solution of ethyl 2-amino-4-methyl-4-pentenoate hydrochloride (as prepared in Example 8) (0.70 g, 3.6 mmol) in CH_2Cl_2 (10 mL) at R. T. under were added *N*-Boc-L-Leu-OH (1.0 g, 4.0 mmol), HOBT

-88-

(1.19 g, 7.9 mmol), EDC (0.76 g, 4.0 mmol) and Et₃N (0.55 mL, 4.0 mmol). The reaction mixture was taken up in additional CH₂Cl₂ (20 mL) and washed with sat. NaHCO₃ (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated. The resulting residue
5 was purified by flash chromatography on silica gel (EA:H; 1:1) to afford the dipeptide ethyl ester as a white solid (1.05 g, 78.6%): ¹H NMR (CDCl₃, 300 MHz) δ 0.92 - 0.97 (m, 6 H), 1.25 - 1.30 (t, 3 H, J = 6 Hz), 1.44 - 1.74 (m, 15 H), 2.37 - 2.57 (m, 2 H), 4.15 - 4.22 (m, 2 H), 4.64 - 4.94 (m, 4 H) ppm.

10 To the above ester (1.0 g, 2.7 mmol) was added 4 N HCl/dioxane (15 mL), stirred at R.T. for 4 h, then the solvent was removed. Co-evaporation with ether (3 x 5 mL) yielded the title compound as a white solid (0.8 g, 96.3%): ¹H NMR (CDCl₃, 300 MHz) δ 0.89 - 1.00 (m, 6 H), 1.21 - 1.31 (m, 3 H), 1.44 - 1.84 (m, 6 H), 2.34 (m, 2 H), 4.16 - 4.75 (m, 4 H) ppm.

15

EXAMPLE 14

Preparation of (2*SR*)-*N*-[(2*S*)-2-benzyloxy-4-methylpentanoyl]-L-Leu *N*-(2-(4-methyl-4-pentenyl))amide

To a stirred solution of L-Leu-OH (5.0 g, 38.2 mmol) in 1 N H₂SO₄ (50 mL) at 0° C was slowly added over 1 1/2 h a solution of sodium nitrite
20 (NaNO₂) (7.5 g, 0.11 mmol) in water (20 mL) while maintaining the temperature at 0° C. The reaction mixture was gradually warmed to R.T., stirred for 24 h, and concentrated to give a white solid. The solid was extracted with ether (5 x 50 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated to give (2*S*)-2-hydroxy-4-
25 methylpentanoic acid as an oil (4.1 g, 81.2%): ¹H NMR (CDCl₃, 300 MHz) δ 0.98 (d, 6 H, J = 12.0 Hz), 1.57 - 1.67 (m, 2 H), 1.82 - 1.93 (m, 1 H), 4.36 (t, 1 H, J = 6.0 Hz) ppm.

To a stirred solution of the acid (4.0 g, 30.5 mmol) in anhydrous DMF (20 mL) at R. T. under Ar was added cesium carbonate (Cs₂CO₃) (12.9 g,
30 40.0 mmol) and methyl iodide (5.7 g, 40.0 mmol). The reaction mixture

-89-

was stirred for 16 h then taken up in EA (100 mL). The organic layer was washed with sat. NaHCO_3 (3 x 20 mL), 1 N HCl (2 x 20 mL), dried (MgSO_4), filtered and concentrated. Purification by flash chromatography on silica gel (EA:H; 1:4) gave methyl (2S)-2-hydroxy-4-methylpentanoate as a colorless oil (2.5 g, 57%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.94 - 1.01 (m, 6 H), 1.56 - 1.74 (m, 2 H), 1.87 - 1.96 (m, 1 H), 3.79 (s, 3 H), 4.24 (q, 1 H, J = 6.0 Hz) ppm.

To a stirred solution of methyl (2S)-2-hydroxy-4-methylpentanoate (0.5 g, 3.4 mmol) in anhydrous CH_2Cl_2 (10 mL) at R.T. under Ar was added benzyl 2,2,2-trichloroacetimidate (1.4 mL, 6.8 mmol) and trifluoromethylsulfonic acid (25 μL). After 30 min the reaction mixture was taken up in CH_2Cl_2 (20 mL). The organic layer was washed with sat. NaCl (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO_4), filtered and concentrated. Purification by flash chromatography on silica gel (EA:H; 1:10) afforded methyl (2S)-2-benzoxy-4-methylpentanoate as an oil (0.6 g, 73.5%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.80 - 0.98 (m, 6 H), 1.40 - 1.58 (m, 1 H), 1.69 - 1.87 (m, 2 H), 3.74 (s, 3 H), 3.93 - 4.06 (m, 1 H), 4.42 (d, 1 H, J = 12.0 Hz), 4.68 - 4.80 (m, 1 H), 7.14 - 7.32 (m, 5 H) ppm.

To the above methyl (2S)-2-benzoxy-4-methylpentanoate (0.69 g, 2.92 mmol) in $\text{MeOH}/\text{H}_2\text{O}$ (5 mL/1 mL) was added $\text{LiOH}\cdot\text{H}_2\text{O}$ (0.28 g, 11.7 mmol) and 30% H_2O_2 (0.3 mL, 11.7 mmol). After stirring the reaction mixture for 24 h, the mixture was treated with 1 N HCl (pH = 3) and the methanol was removed *in vacuo*. The aqueous layer was extracted with EA (4 x 15 mL). The combined organic layers were washed with 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO_4), filtered and concentrated. (2S)-2-benzoxy-4-methylpentanoic acid was isolated as a colorless oil (0.65 g, 100%): ^1H NMR (CDCl_3 , 300 MHz) δ 0.82 - 1.60 (m, 6 H), 1.53 - 1.62 (m, 1 H), 1.73 - 1.90 (m, 2 H), 3.99 - 4.50 (m, 1 H), 4.46 (d, 1 H, J = 12.0 Hz), 4.72 (d, 1 H, J = 12.0 Hz) 7.10 - 7.26 (m, 5 H) ppm.

-90-

To a solution of the product from Example 13 (0.5 g, 1.63 mmol) in anhydrous CH_2Cl_2 (10 mL) at R.T. under Ar was added the above acid (0.4 g, 1.8 mL), HOBT (0.24 g, 1.8 mmol), EDC (0.35 g, 1.8 mmol) and Et_3N (0.25 mL, 1.8 mmol). After 16 h the reaction mixture was washed with
5 sat. NaHCO_3 (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL) dried (MgSO_4), filtered and concentrated. Purification by flash chromatography afforded the ethyl ester (0.5 g, 62.5%): Reporting a mixture of diastereomers ^1H NMR (CDCl_3 , 300 MHz) δ 0.76 (t, 3 H, $J = 6.0$ Hz), 0.91 - 0.99 (ol-m, 12 H), 1.22 - 1.9 (ol-m, 9 H), 2.05 - 2.18 (ol-m, 1 H), 2.34 -
10 2.43 (ol-m, 1 H), 3.71 - 4.95 (ol-m, 9 H), 7.20 - 7.38 (ol-m, 5 H) ppm.

To a solution of the ester (0.5 g, 1.0 mmol) in anhydrous THF (10 mL) at R.T. under Ar was added LiBH_4 (0.02g, 1.0 mmol). Reaction mixture was stirred for 4 h then quenched by the addition of 1 N HCl (1 mL), concentrated, and extracted with EA (3 x 10 mL). The combined organic
15 layers were washed with sat. NaHCO_3 (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO_4), filtered and concentrated. Purification by flash chromatography on silica gel (EA:H; 1:3) gave the alcohol as an oil (0.13 g, 30%): Reporting a mixture of diastereomers ^1H NMR (CDCl_3 , 300 MHz) δ 0.72 - 0.98 (ol-m, 2 H), 1.10 - 2.21 (ol-m, 11 H), 3.58 - 4.92 (ol-
20 m, 9H), 7.0 - 7.50 (ol-m, 7 H) ppm.

To anhydrous DMSO (51.1 μl , 0.72 mmol) in anhydrous THF (10 mL) at -78°C under Ar was slowly added oxalyl chloride (39.3 μl , 0.45 mmol). The mixture was stirred at -78°C under Ar for 30 min. The alcohol (0.13 g, 3.0 mmol) in anhydrous THF (5 mL) was slowly added, and stirred for an
25 additional 1 1/2 h. Et_3N (0.18 mL, 1.4 mmol) was then added, and mixture was gradually warmed to R.T. Stirring was continued for 2 h at R.T. The reaction mixture was taken up in EA (50 mL). The organic layer was washed with sat. NaCl (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaHCO_3 (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO_4), and concentrated. Purifica-
30 tion by flash chromatography on silica gel (EA:H; 1:4) afforded the desired

-91-

title aldehyde (0.13 g, 99%): Reporting a mixture of diastereomers ^1H NMR (CDCl_3 , 300 MHz) δ 0.72 - 0.91 (ol-m, 12 H), 1.10 - 2.30 (ol-m, 11 H), 4.0 - 5.01 (ol-m, 7 H), 7.0 - 7.30 (ol-m, 7 H), 9.8 + 9.76 (s+s, 1 H) ppm.

EXAMPLE 15**5 Preparation of (2SR)-(3S)-N-Cbz-L-Leu-Leu N-[3-(2-hydroxy-heptanoic acid)]amide**

To a stirred solution of *N*-Boc-L-Nle *N*-methoxy-*N*-methylamide (1.0 eq) in anhydrous THF (10 mL) at 0° C under Ar is added LiAlH_4 (1.0 eq), and stirred for 3 h at 0° C followed by the addition of 1 N HCl (1 mL). The
10 mixture is taken up in EA then washed with sat. NaHCO_3 , 1 N HCl, sat. NaCl, dried (MgSO_4), filtered and concentrated to afford the *N*-Boc-L-norleucinal as a crude residue. To the residue is added an ice-cold solution of NaHSO_3 (8 eq) and the mixture is stirred for 24 h at 5° C. To the resulting suspension is added EA and an aqueous potassium cyanide solution
15 (KCN) (8 eq). The reaction mixture is stirred at R.T. for 4 h. The organic phase is washed with water and concentrated to give the cyanohydrin.

The cyanohydrin is hydrolyzed in 4 N HCl/dioxane under reflux for 12 h. The solvent is removed and the residue is washed with anhydrous ether to give the hydrolyzate. To a stirred solution of *N*-Cbz-L-Leu-Leu-OH (1.0
20 eq) in anhydrous CH_2Cl_2 under Ar at R.T. is added CDI (1.1 eq). After 30 min of stirring Et_3N (2 eq) and the hydrolyzate (1.0 eq) is added. The mixture is stirred for 6 h, then concentrated. The residue is triturated with 1 N HCl washed with water and dried *in vacuo* to afford the title compound.

EXAMPLE 16**25 Preparation of (2SR)-(3S)-N-Cbz-L-Leu-L-Leu N-[3-(methyl 2-hydroxy-heptanoate)]amide**

To the product obtained from Example 15 in anhydrous ether at 0° C is added diazomethane. After 3 h the solvent is removed and the residue is purified by flash chromatography on silica gel to give the desired product.

-92-

EXAMPLE 17

Preparation of (2*SR*)-(3*S*)-*N*-Cbz-L-Leu-L-Leu *N*-[3-(benzyl 2-hydroxy-heptamide)]amide

- To the product of Example 15 in anhydrous CH₂Cl₂ under Ar at R.T.
- 5 is added HOBT (1.0 eq), EDC (1.0 eq), Et₃N (1.0 eq) and benzylamine (1.0 eq). After 6 h the reaction mixture is washed with sat. NaHCO₃, sat. 1 N HCl, sat. NaCl, dried (MgSO₄), filtered and concentrated. The residue is purified by flash chromatography on silica gel to afford the desired product.

EXAMPLE 18

- 10 Preparation of (3*SR*)-(4*S*)-*N*-Cbz-L-Leu-L-Leu *N*-[4-(benzyl 3-hydroxy-octamide)]amide

- To a solution of *N*-Boc-L-norleucinal (1.0 eq), prepared by reducing *N*-Boc-L-Nle *N*-methoxy-*N*-methylamide as described in Example 15, in THF at -78° C under Ar is added ethyl lithioacetate (2.2 eq) prepared *in situ* by the
- 15 addition of *n*BuLi (2.2 eq) to excess anhydrous ethyl acetate. After 3 h, the reaction mixture is treated with 1 N HCl, and the organic layer is washed with 1 N HCl, sat. NaHCO₃, sat. NaCl, dried (Mg SO₄), filtered and concentrated. Purification by flash chromatography on silica gel gives the ester.

- The ester is treated with 4 N HCl/dioxane for 30 min, and the
- 20 concentrated *in vacuo*. The resulting solid is taken up in anhydrous ether and concentrated *in vacuo* to give the hydrochloride. The hydrochloride is used without further purification in the next step.

- To the hydrochloride (1.0 eq) in anhydrous CH₂Cl₂ at R.T. under Ar is added HOBT (2.0 eq), EDC (1.0 eq), Et₃N (1.0 eq) and *N*-Cbz-L-Leu-Leu-
- 25 OH (1.1 eq). After 6 h, the organic is washed with sat. NaHCO₃, 1 N HCl, dried (MgSO₄), filtered and concentrated. Purification by flash chromatography on silica gel gives the ester.

- To a stirred solution of the above ester (1.0 eq) in MeOH-H₂O is added LiOH.H₂O (2 eq) and H₂O₂ (1.0 eq). After 4 h the reaction is
- 30 quenched by the addition of 10% HCl and then extracted with EA (2 x).

-93-

The combined organic layers are washed with sat. NaCl, dried (MgSO₄) and concentrated to give the acid.

To a solution of the acid (1.0 eq) in anhydrous CH₂Cl₂ at R.T. under Ar is added EDC (1.0 eq), HOBT (1.0 eq), Et₃N (1.0 eq) and benzylamine
5 (1.1 eq). The reaction mixture is stirred for 3 h, washed with sat. NaHCO₃,
1 N HCl, sat. NaCl, dried (MgSO₄), filtered and concentrated. Purification
by flash chromatography on silica gel affords the title compound.

EXAMPLE 19

Preparation of (3S)-N-Cbz-L-Leu-L-Leu N-[3-(1-furfylthio-2-oxo-
10 heptane)amide

To the N-Boc-L-Nle-CHN₂ (1.0 eq) is added HCl(g) and pyridine (5 mL).
The mixture is taken up in EA and washed with 1 N HCl, sat. NaHCO₃, sat.
NaCl, dried (MgSO₄), filtered and concentrated to give the chloromethyl
ketone.

15. To a solution of the chloromethyl ketone (1.0 eq) in anhydrous THF
under Ar at R.T. is added furfuryl mercaptan (2.0 eq) and Et₃N (2.0 eq). The
reaction mixture is stirred at R.T. for 16 h, and extracted with EA. The
combined organic layers are washed with sat. NaCl, sat. NaHCO₃, 1 N HCl,
dried (MgSO₄), filtered and concentrated. Purification by flash chroma-
20 tography on silica gel affords the furfurylthio-derivative. The derivative is
treated with 4 N HCl/dioxane to yield the hydrochloride salt.

To a solution of the above salt (1.0 eq) in anhydrous CH₂Cl₂ at R.T.
under Ar is added HOBT (2.0 eq), EDC (1.0 eq), Et₃N (1.0 eq) and N-Cbz-L-
Leu-L-Leu-OH (1.1 eq). After 6 h the reaction mixture is washed with sat.
25 NaHCO₃, 1 N HCl, sat. NaCl, dried (MgSO₄) filtered and concentrated.
Purification by flash chromatography on silica gel gives the title compound.

-94-

EXAMPLE 20

Preparation of (2*S*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[2-(4-methyl-4-pentenal)]amide

To a stirred solution of 4-methylvaleric acid (10.8 mL, 86.1 mmol) in
5 anhydrous CH₂Cl₂ (25 mL) was added thionyl chloride (25 mL, 0.34 mmol).
The mixture was placed under reflux for 24 h. Then solvent and excess
thionyl chloride was removed *in vacuo* to give the acid chloride as an oil
(10.2 g, 90%). The acid chloride was used directly in the next step.

To the acid chloride (2.7 g, 20.3 mmol) in anhydrous CH₂Cl₂ (50 mL)
10 at R.T. under Ar was added DMAP (0.10 g), Et₃N (4.6 mL, 33.8 mmol) and
(4*S*, 5*R*)-(-)-4-methyl-5-phenyl-2-oxazolidinone (3.0 g, 16.9 mmol). The
reaction mixture was stirred for 16 h then washed with 1 N HCl (2 x 10
mL), sat. NaHCO₃ (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO₄),
filtered and concentrated. Purification by flash chromatography on silica gel
15 (EA:H; 1:20) afforded the imide as an oil (2.8 g, 61%): ¹H NMR (CDCl₃,
300 MHz) δ 0.80-1.10 (m, 9 H), 1.60-1.90 (m, 3 H), 2.40-2.55 (m, 2 H),
4.80-4.90 (m, 1 H), 5.70-5.80 (m, 1 H), 7.20-7.50 (m, 5 H) ppm.

To a solution of the imide (2.5 g, 9.14 mmol) in anhydrous THF (40
mL) at -78° C under Ar was slowly added a 1.5 M solution of lithium
20 diisopropylamide (LDA) in anhydrous THF (6.0 mL, 9.14 mmol) followed by
cinnamyl bromide (1.8 g, 9.14 mL) in anhydrous THF (10 mL). The reaction
mixture was stirred at -78° C for 1 h then gradually warmed to R.T. Stirring
was continued at R.T. for 1 h then the mixture was treated with 1 N HCl (5
mL). The solvent was removed and the aqueous was taken up in EA (70
25 mL). The aqueous was separated and the organic was washed with sat.
NaCl (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaHCO₃ (2 x 10 mL), sat. NaCl
(2 x 10 mL), dried (MgSO₄) filt red and concentrated. Purification by flash
chromatography on silica gel (EA:H; 1:4) afforded the alkylated derivative
as an oil (3.5 g, 98.7%): ¹H NMR (CDCl₃, 300 Mhz) δ 0.73 (d, 3 H, *J* = 6.0
30 Hz), 0.80-0.92 (m, 6 H), 1.14-1.40 (m, 1 H), 1.48-1.79 (m, 1 H), 1.75-

-95-

1.84 (m, 1 H), 2.42 (m, 2 H), 4.10-4.20 (m, 1 H), 4.71-4.78 (m, 1 H), 5.28 (d, 1 H, $J = 9.0$ Hz), 6.35 (m, 1 H), 6.45 (m, 1 H), 7.21-7.25 (m, 5 H) ppm.

To a solution of the above product (0.65 g, 1.66 mmol) in 3:1
5 MeOH:H₂O (20 mL) at R.T. was added LiOH.H₂O (0.61 g, 4.98 mmol) and
30% H₂O₂ (0.83 mL). The reaction mixture was stirred for 4 h. The
mixture was cooled to 0° C and quenched by the addition of 1 M Na₂S₂O₃
(1.6 mL) and allowed to warm to R.T. After 14 h the resulting solution was
poured onto sat. NaHCO₃ (20 mL). The aqueous was extracted with CH₂Cl₂
10 (3 x 30 mL) then acidified with 1 N HCl (pH = 3). The aqueous was then
extracted with CH₂Cl₂ (3 x 20 mL) and the combined organics were dried
(MgSO₄), filtered and concentrated. The residue was purified by flash
chromatography on silica gel (EA:H; 1:1) to give (2S)-2-(1'-phenyl-
1'-propene)-4-methylpentanoic acid as an oil (0.30 g, 78.0%): ¹H NMR
15 (CDCl₃, 300 MHz) δ 0.88-1.01 (m, 6 H), 1.29-1.42 (m, 3 H), 1.59-1.70
(m, 2 H), 2.55 (m, 3 H), 6.13 (m, 1 H), 6.47 (d, 1 H, $J = 6.0$ Hz), 7.31-
7.40 (m, 5 H) ppm.

To a stirred solution of L-Leu-OMe HCl (0.14 g, 0.70 mmol) and (2S)-
2-(1'-phenyl-1'-propene)-4-methylpentanoic acid (0.16 g, 0.69 mmol) in
20 anhydrous CH₂Cl₂ (15 mL) at R.T. were added HOBt (0.19 g, 1.38 mmol),
EDC (0.15 g, 0.78 mmol) and Et₃N (0.91 mL, 0.70 mmol). After 16 h the
reaction mixture was washed with sat. NaHCO₃ (2 x 10 mL), 1 N HCl (x 10
mL), sat. NaCl (2 x 10 mL), dried (MgSO₄), filtered and concentrated.
Purification of the crude residue by flash chromatography on silica gel (EA:H;
25 1:1) afforded the methyl ester as an oil (0.24 g, 95%): ¹H NMR (CDCl₃,
300 MHz) δ 0.76 - 0.78 (d, 3 H, $J = 6.0$ Hz), 0.82 - 1.06 (m, 3 H), 1.26 -
1.41 (m, 1 H), 1.44 - 1.72 (m, 2 H), 2.22 - 2.30 (m, 2 H), 2.35 - 2.46 (m,
1 H) 3.72 (s, 3 H), 4.57 (m, 1 H), 6.16 (m, 1 H), 6.43 (m, 1 H), 7.25 -
7.41 (m, 5 H) ppm.

-96-

To the above methyl ester (0.28 g, 0.78 mmol) in MeOH-H₂O (15 mL-
5 mL) with stirring at 0° C was added 1 N LiOH.H₂O (0.95 mL) and 30%
H₂O₂ (1.4 mL). After 2 h 1 N HCl (4 mL) was added and the aqueous layer
was extracted with EA (3 x 30 mL). The combined organics were washed
5 with sat. NaCl (2 x 20 mL), dried (MgSO₄), filtered and concentrated to give
a crude residue. Purification by flash chromatography on silica gel (EA:H;
1:1) afforded the acid as an oil (0.21g, 0.61 mmol): ¹H NMR (CDCl₃, 300
MHz) δ 0.59 - 1.10 (m, 12 H), 1.12 - 1.71 (m, 6 H), 2.60 - 2.63 (m, 3 H),
4.45 (m, 1 H), 6.21 (m, 1 H) 6.40 (m, 1 H), 7.25 - 7.41 (m, 5 H) ppm.

10 To a stirred solution of the acid (0.25 g, 0.72 mmol) and ethyl 2-
amino-4-methyl-4-pentenoate (0.16 g, 0.8 mmol) in anhydrous CH₂Cl₂ (15
mL) at R.T. were HOBt (0.19 g, 1.44 mmol), EDC (0.15 g, 0.79 mmol) and
Et₃ N (0.12 mL, 0.80 mmol). After 4 h the reaction mixture was washed
with sat. NaHCO₃ (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10mL),
15 dried (MgSO₄), filtered and concentrated. The residue was purified by flash
chromatography on silica gel (EA:H; 1:1) to (2*SR*)-[(2*R*)-[2-(1'-phenyl-1'-
propene)-4-methyl pentanoyl]]-L-Leu *N*-[2-(ethyl 4-methyl-4-penteno-
ate)]amide as a solid (0.18g, 51%): ¹H NMR CDCl₃, 300 MHz) δ 0.81 -
1.10 (m, 12 H), 1.30 - 1.73 (m, 15 H), 2.27 - 2.51 (m, 2 H), 4.14 - 4.17
20 (m, 2 H), 4.22 - 4.49 (m, 2 H), 4.59 (m, 1 H), 6.21 (m, 1 H), 6.43 (m, 1 H),
7.17 - 7.19 9m, 2 H), 7.25 - 7.41 (m, 5H) ppm.

To the above ethyl ester (0.3 g, 0.62 mmol) in anhydrous THF (20
mL) at 0° C under Ar with stirring was added LiBH₄ (47 mg, 2.15 mmol).
After 30 min at 0° C the reaction mixture was warmed to R.T. Stirring was
25 continued for 2 h then quenched with 1 N HCl (2mL). The mixture was
extracted with EA (3 x 10 mL). The combined organics were washed with
sat. NaHCO₃ (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried
(MgSO₄), filtered and concentrated. Purification of the crude by flash
chromatography on silica gel (EA) to give the alcohol as an oil (0.24 g,
30 87%): ¹H NMR (CDCl₃, 300 MHz) δ 0.72 - 1.02 (m, 12 H), 1.20 - 1.86 (m,

-97-

13 H), 2.21 - 2.56 (m, 4 H), 3.60 - 3.68 (m, 2 H), 4.21 - 4.25 (m, 1 H), 4.80 - 4.85 (m, 1 H), 6.10 - 6.15 (m, 1 H), 6.45 - 6.50 (m, 1 H), 7.17 - 7.19 (m, 2 H), 7.25 - 7.35 (m, 5 H) ppm.

To anhydrous DMSO (0.08 mL, 1.08 mmol) in anhydrous CH_2Cl_2 (5 mL) at -78°C under Ar with stirring was added oxalyl chloride (0.06 mL, 0.68 mmol). The mixture was stirred for 30 min then the alcohol (0.19 g, 0.45 mmol) in anhydrous CH_2Cl_2 (5 mL) was added. Stirring was continued at -78°C for 2 h. Et_3N (0.29 mL) was then added to the reaction mixture and the mixture was allowed to gradually warm to R.T. Stirring was continued for 1 h. The reaction mixture was taken up in additional CH_2Cl_2 (20 mL). The organic was washed with sat. NaHCO_3 (2 x 10 mL), 1 N HCl (2 x 10 mL), sat. NaCl (2 x 10 mL), dried (MgSO_4), filtered and concentrated to give a crude residue. Purification by flash chromatography on silica gel (EA:H;1:1) afforded the title compound as an oil (20.0 g, 10%): Reporting a mixture of diastereomers ^1H NMR (CDCl_3 , 300 MHz) δ 0.78 - 1.79 (ol-m, 23 H), 1.96 - 2.66 (ol-m, 2 H), 4.11 - 4.93 (ol-m, 4 H), 5.77 - 6.43 (ol-m, 3 H), 7.19 - 7.25 (ol-m, 5 H), 7.97 - 8.06 (ol-m, 2 H), 9.56 + 9.57 (s + s, 1 H) ppm.

EXAMPLE 21

20 Preparation of *N*-Cbz-L-Pro-L-Leu-L-norleucinal

The title compound was isolated as a colorless oil (0.13 g) using the methodology outlined in example 2: ^1H NMR (CDCl_3 , 300 MHz) δ 0.80 - 0.91 (m, 9 H), 1.24 - 2.22 (m, 3 H), 3.45 - 3.60 (m, 2 H), 4.11 - 4.43 (m, 3 H), 5.06 - 5.16 (m, 2 H), 6.40 - 7.00 (m, 2 H), 7.30 - 7.40 (m, 5 H), 9.5 (s, 1 H) ppm.

-98-

EXAMPLE 22

Preparation of *N*-Cbz-L-Leu-L-Leu-DL-cyclohexylglycinal

Using the procedure set forth in Example 3 the title compound was isolated as a yellowish solid (50.0 mg): Reporting a mixture of
5 diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.87 - 1.72 (ol-m, 29 H), 1.99 + 2.00 (s + s, 3 H), 4.35 - 4.75 (ol-m, 3 H), 6.57 - 7.82 (ol-m, 3 H), 9.56 - 9.63 (ol-m, 1 H) ppm.

EXAMPLE 23

Preparation of *N*-Fmoc-L-Leu-L-Leu-DL-norleucinal

10 The title compound was prepared from the corresponding propane thiol ester using substantially the same procedure described in Example 3. *N*-Fmoc-L-Leu-L-Leu-DL-norleucinal was isolated as a colorless oil (15 mg): Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.52 - 0.61 (ol-m, 3 H), 0.80 - 1.05 (ol-m, 16 H), 1.22 - 1.26 (ol-m, 2 H), 2.02 -
15 2.10 (ol-m, 4 H), 4.04 - 4.69 (ol-m, 5 H), 5.21 - 5.27 (ol-m, 1 H), 7.23 - 7.84 (ol-m, 8 H), 9.50 - 9.56 (ol-m, 1 H) ppm.

EXAMPLE 24

Preparation of (2*SR*)-*N*-Ac-L-Leu-L-Leu *N* [2-(*trans*-4-hexenal)]amide

The title compound isolated as a white solid (0.48 g) following the
20 procedure set forth in Example 9: Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.89 - 1.26 (ol-m, 12 H), 1.51 - 1.74 (ol-m, 9 H), 1.93 (s, 3 H), 2.40 - 2.61 (ol-m, 2 H), 3.40 - 4.01 (ol-m, 0.3 H), 4.35 - 4.61 (ol-m, 3 H), 5.30 - 5.37 (ol-m, 1 H), 5.49 - 5.50 (ol-m, 1 H), 6.70 - 7.71 (ol-m, 3 H), 9.50 - 9.56 (ol-m, 0.7 H) ppm.

25

EXAMPLE 25

Preparation of *N*-Ac-L-Leu-L-Phe-DL-norleucinal

N-Ac-L-Leu-L-Leu-DL-norleucinal was isolated as a white solid (0.12 g) using similar methodology that is set forth in Example 3: Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.81 - 0.88 (ol-m, 9
30 H), 1.23 - 1.83 (ol-m, 8 H), 1.94 - 1.96 (s + s, 3 H), 2.96 - 3.21 (ol-m, 0.4

-99-

H), 3.31 - 3.47 (ol-m, 2 H), 4.02 - 5.10 (ol-m, 3 H), 7.05 - 7.20 (ol-m, 5 H)
9.20 - 9.49 (ol-m, 0.6 H) ppm.

EXAMPLE 26**Preparation of *N*-Cbz-L-Leu-L-Leu-L-norleucinal**

- 5 Using the procedure set forth in Example 2 the title compound was isolated as a colorless oil (0.22 g): ¹H NMR (CDCl₃, 200 MHz) δ 0.86 - 0.93 (m, 9 H), 1.23 - 1.86 (m, 9 H), 2.37 (m, 0.2 H), 3.90 (s, 2 H), 4.39 - 4.51 (m, 1 H), 4.57-4.61 (m, 1 H), 5.08 (m, 2 H), 5.94 (hs, 1 H), 7.12 - 7.23 (m, 2 H), 7.23 - 7.32 (m, 5 H), 9.49 (s, 0.8 H) ppm.

10

EXAMPLE 27**Preparation of (2*SR*)-*N*-Ac-L-Leu-L-Leu *N*-[2-(4-methyl-4-pentenall)]amide**

- The title compound was isolated as a white solid (0.2 g) following substantially the same procedure described in Example 9: Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.81 - 0.88 (ol-m, 12
15 H), 1.31 - 1.90 (ol-m, 8 H), 1.92 (ol-m, 3 H) 2.23 - 2.56 (ol-m, 2 H), 4.70 - 4.80 (ol-m, 1 H), 4.78 - 5.00 (ol-m, 3 H), 7.70 - 7.80 (ol-m, 1 H), 8.01 - 8.60 (ol-m, 2 H), 9.43 + 9.52 (s + s, 1 H) ppm.

EXAMPLE 28**Preparation of *N*-Cbz-L-Leu-L-(methyl)Leu-DL-norleucinal**

- 20 Following the procedures set forth in Examples 1 and 2 the title compound was isolated as a colorless oil (0.14 g): Reporting a mixture of diastereomers ¹H NMR (CDCl₃, 300 MHz) δ 0.86 - 1.02 (ol-m, 15 H), 1.26 - 1.98 (ol-m, 12 H), 2.80 - 3.00 (ol-s, 3 H), 4.30 - 4.80 (ol-m, 3 H), 4.95 - 5.20 (ol-m, 2 H), 7.29 - 7.36 (ol-m, 5 H), 9.51 (ol-s, 1 H) ppm.

25

EXAMPLE 29**Pr eparation of *N*-Dansyl-L-Leu-L-Leu-DL-n rl eucinal**

- The title compound was isolated as a pale yellow solid (0.13 g) using the methodology described in Example 9: Reporting a mixture of diast reomers ¹H NMR (CDCl₃, 300 MHz) δ 0.58 - 2.50 (ol-m, 23 H), 2.89
30 (ol-s, 6 H), 3.30 - 3.70 (ol-m, 1 H), 4.38 - 4.52 (ol-m, 2 H), 4.81 - 4.94 (ol-

-100-

m, 1 H), 5.30 - 5.71 (ol-m, 1 H), 6.75 - 6.96 (ol-m, 1 H), 7.19 - 7.36 (ol-m, 2 H), 7.50 - 7.65 (ol-m, 2 H), 8.20 - 8.30 (ol-m, 2 H), 8.56 - 8.61 (ol-m, 1 H), 9.52 - 9.61 (ol-s, 1 H) ppm.

EXAMPLE 30**5 Preparation of *N*-Ac-L-Phe-L-Leu-DL-norleucinal**

The title compound *N*-Ac-L-Phe-L-Leu-DL-norleucinal was obtained as a white solid (0.19 g) following substantially the same procedure described in Example 9: Reporting a fully hydrated amino-aldehyde ¹H NMR (CDCl₃, 300 MHz) δ 0.64 - 1.01 (ol-m, 9 H), 1.51 - 1.71 (ol-m, 9 H), 1.87 - 1.95
10 (ol-s, 3 H), 2.79 - 3.17 (ol-m, 2 H), 3.34 - 3.41 (ol-s, 3 H), 3.85 - 4.68 (ol-m, 4 H), 7.16 - 7.29 (ol-m, 5H) ppm.

EXAMPLE 31**ASSAYS FOR IDENTIFICATION OF COMPOUNDS HAVING ACTIVITY AS MODULATORS OF THE PROCESSING OF APP****15 A. Immunoblot assay for Aβ peptide**

Human glioblastoma cells (ATCC Accession No. HTB16) were stably transfected with a DNA expression vector encoding a 695 amino acid isoform variant of the amyloid precursor protein (APP) containing the familial Swedish double mutations at codons 670 and 671 (K to N and M to L,
20 respectively; see Mullan et al. (1992) Nature Genet. 1:345-347) and an additional mutation at codon 717 (V to F; see Murrell et al. (1991) Science 254:97-99) to produce cells designated HGB 717/Swed. High levels of Aβ are detectable in the conditioned medium isolated from HGB 717/Swed cultured cells. The medium also contains larger secreted fragments, α-
25 sAPP₆₉₅, which are alternatively processed APP fragments whose generation precludes Aβ formation.

HGB 717/Swed cells were grown at 37°C under a 5% carbon dioxide atmosphere in Dulbecco's modified eagle medium (DMEM; Gibco) supplemented with 10% heat-inactivated fetal calf serum, 0.45% glucose,
30 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin

-101-

sulfate (Gemini Bioproducts). Approximately 1×10^6 cells were incubated overnight in 5 ml of DMEM containing varying μ M final concentrations of desired test compounds or a DMSO control. Conditioned medium was collected, and unwanted cells and debris were removed by sedimentation at 3,000 rpm at 4°C.

A β peptides were isolated from the medium by immunoaffinity purification using an A β -specific antibody. To reduce the interaction of non-specific binding of unrelated proteins, such as serum proteins, to the A β antibody, the medium was pre-treated with rabbit antisera and Protein A Sepharose (Pharmacia) for 4 hours at 4°C. The Sepharose-bound material was removed by centrifugation at 3,000 rpm at 4°C for 10 min, and A β peptides were immunoaffinity purified from the clarified medium by incubation overnight with an affinity purified polyclonal rabbit antibody (referred to as 2939) prepared against a synthetic A β peptide corresponding to amino acids 1 to 28. Protein A-conjugated Sepharose was added to immobilize the A β -antibody complexes, and the resin was pelleted by centrifugation at 3,000 rpm at 4°C for 10 min. The A β -antibody complexes were eluted from the matrix by denaturing the complex by boiling in the presence of SDS.

Equal portions of each sample were loaded on 16% Tricine gels (Novex), and subjected to electrophoresis. Resolved proteins were transferred from the gel to Hybond nitrocellulose (Amersham, Arlington Heights, IL) by electroblotting, and incubated with the commercially available monoclonal antibody 6E10 (obtained from Drs. Kim and Wisniewski, Institute for Basic Research, NY, see, published International PCT application WO 90/12871), which specifically recognizes A β residues 1 to 17. Specifically bound antibody was detected using a biotinylated goat anti-mouse IgG secondary antibody (Sigma), followed by the addition of streptavidin conjugated to horseradish peroxidase (Amersham, Arlington Heights, IL), and visualized by luminescent detection (Amersham). Levels of A β peptides

-102-

were determined by laser densitometry of visualized films. A positive result in the assay is a decrease in the formation of the 4-kDa A β peptides in cells treated with test compound relative to the DMSO control. The values for the relative percent inhibition of A β formation for several of the compounds

5 are shown in Table I:

INHIBITION OF A β FORMATION	
COMPOUND	% INHIBITION OF A β
Ac-L-L-NI-(DM)	87 ^a
10 Cbz-L-L-[CH ₃]-NI-(H)	97 ^b
Ac-L-L-NI-(CN)	94 ^a
Cbz-L-F-Lene-(H)	69 ^c
Ac-L-L-Chg-(H)	99 ^a
Cbz-L-F-Cha-(H)	100 ^b
15 Ac-L-L-Cha-(H)	46 ^c
Ac-L-L-Nlene-(H)	96 ^a
Cbz-P-L-NI-(H)	80 ^b
Cbz-L-L-Lene-(H)	95 ^c
Cbz-L-[CH ₃]-L-NI-(H)	77 ^b
20 2S-benzoxysvaleric-L-Lene-(H) ^a	85 ^b
Ac-L-L-NI-(TFMK)	50 ^a
Cbz-P-L-Lene-(H)	40 ^b
25 Fmoc-L-L-NI-(H)	89 ^a

^a Inhibition of A β formation was determined at a concentration of 75 μ M.

^b Inhibition of A β formation was determined at a concentration of 40 μ M.

^c Inhibition of A β formation was determined at a concentration of 10 μ M.

-103-

- Inhibition of A β formation was determined at a concentration of 50 μ M.

5 • dipeptide
NI norleucine
Lene 2-amino-4-methyl-4-pentenoic acid (unsaturated *iso*-butyl leu side chain)

10 NIene 2-amino-4-hexenoic acid (unsaturated n-butyl Nle side chain)

(H) aldehyde

(TFMK) trifluoromethylketone

15 (DM) diazomethylketone

(CN) nitrile

B. ELISA assay for total sAPP

20 Human glioblastoma cells (ATCC Acession No. HTB16) were stably transfected with a DNA expression vector encoding the 695 amino acid isoform of the amyloid precursor protein (APP₆₉₅). The resulting cells are designated HGB695 cells. High levels of secreted proteolytic processed fragments of APP₆₉₅ (sAPP₆₉₅) are detectable in the culture medium.

25 Approximately 1 X 10⁵ cells were plated into 12-well dishes and were grown for 72 hours at 37°C under a 5% carbon dioxide atmosphere in 1 ml of Dulbecco modified eagle medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 0.45% glucose, and 100 units/ml penicillin, 100 μ g/ml streptomycin sulfate and 2 mM L-glutamine. Following incubation,
30 the medium was removed and 1 ml of supplemented DMEM medium containing 5 μ l of DMSO or DMSO containing the desired test compound within a range about 5 to 100 μ M (final concentration in the well), was added to each well, and incubation was continued for 24 hours. Unwanted cells and debris were removed by sedimentation at 3,000 x g for 10 min at
35 room temperature. Supernatants wer stored at -20°C for analysis.

-104-

In order to determine the amount of sAPP in the supernatants, a capture antibody which recognizes an epitope located in the amino terminus of APP, such as monoclonal antibody P2-1 (see, e.g., U.S. Patent No. 5, 270,165), or a goat affinity-purified IgG antibody raised against wildtype sAPP₆₉₅, was attached to the wells of a 96-well plate by incubating the antibody in the plate for 1-2 hr at 37°C. The plates were washed three times with 0.3 ml of 0.1% Tween-20 in phosphate-buffered saline (PBS). The non-specific interaction of unrelated proteins (such as serum proteins that may interfere with the analysis) with the antibody was reduced by incubating the pre-treated wells for 30 min at 37 ° C with a solution of 0.5% casein or 0.5% gelatin in PBS (150-200 µl/well). Wells were washed thoroughly with 0.1% Tween-20 in PBS prior to analysis of samples.

The conditioned medium supernatant was diluted 1:20 in 0.95 ml of 0.1% CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate) in PBS. Supernatant samples (50 or 100 µl/well) or sAPP standards (50 or 100 µl/well) of a range about 5 to 50 ng/ml were added to the pre-treated wells and incubated for 1-2 hr at 37°C. The supernatant was removed and each sample well was washed as described above. A horseradish peroxidase (HRP) conjugated goat affinity purified antibody, raised against sAPP₆₉₅, was diluted in 0.1% Tween-20 in PBS and 10% goat serum and employed as the "signal antibody". The unbound antibody was removed by washing, and to each well, 0.1 ml of the chromagenic substrate K-Blue Solution (Elisa Technologies, Lexington, KY) was added and samples were incubated in the absence of visual light for 15 min at ambient temperature. Reactions were stopped by the addition of 0.1 ml of a 9.8% solution of phosphoric acid. The optical density of samples was measured by spectrophotometry at 450 nm. The concentration of sAPP₆₉₅ peptid s in the conditioned medium was estimated from the sAPP₆₉₅ standard curve. Samples were analyzed in duplicate with the appropriate standards and

-105-

reference controls [i.e., a known protease inhibitor compound, such as N-acetyl-leucyl-leucyl-norleucinal of given potency and concentration].

C. CELL LYSATE ASSAY

In this assay, the effect of compounds on the modulation of the generation of partially processed C-terminal A β -containing amyloidogenic peptides is examined. HGB695 human glioblastoma cells were employed and grown in 12-well dishes essentially as described in Example 31B with the following modifications. The DMEM growth media were supplemented with varying μ M concentrations of compounds or DMSO control and 100 μ M leupeptin and 1 μ M PMA phorbol ester and were incubated with cell cultures for 16 hours and cells were grown to approximately 2.5×10^6 cells per well.

Harvested cells from each well were lysed in 100 μ l of lysis buffer containing 50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 1% NP-40, 0.1% SDS and 0.5% deoxycholate supplemented with 1 mM PMSF. Equal volumes of cell lysates in Laemmli SDS buffer were loaded onto 16% SDS-Tricine polyacrylamide gels (Novex) and subjected to electrophoresis. Separated proteins were transferred to supported nitrocellulose (BioRad) by electroblotting. Nonspecific binding of proteins to the nitrocellulose membrane was blocked by incubating in a solution of 5% non-fat dried milk in PBS. The nitrocellulose membrane was washed three times in PBS and then incubated in PBS containing a 1:5000 dilution of a rabbit polyclonal antibody raised against the C-terminal 19 amino acids of APP (provided by S. Gandy, Rockefeller University, NY). The nitrocellulose membrane was washed as described above and incubated with a secondary biotinylated goat anti-rabbit IgG antibody. Specifically bound antibody was detected using a streptavidin horseradish peroxidase conjugate, and visualized in combination with an enhanced chemiluminescent detection kit (Amersham). Potentially amyloidogenic peptides greater than 9 and less than 22 kDa were quantitated by densitometric scans of developed films within the linear range

-106-

as described in Example 31B. A positive result for a compound in the cell lysate assay is denoted by a decrease in the levels of the protein bands greater than 9 and less than 22 kDa relative to the appropriate control samples.

5 D. ELISA ASSAY FOR α -sAPP

Human HGB695 glioblastoma cells transfected with DNA encoding the 695 amino acid isoform of APP were grown and treated with test compound or DMSO as described in Example 31B. Media from the cultured cells were obtained also as described in Example 31B and analyzed for α -

10 sAPP in an ELISA assay as follows.

The wells of a 96-well microtiter plate were coated with a monoclonal antibody that specifically recognizes the amino terminus of human sAPP (e.g., monoclonal antibody P2-1) or with a rabbit polyclonal antibody (designated 3369) generated against a peptide including A β amino acid
15 residues 1-15 by incubating the antibody in the plate for 60 min at 37°C. The plates were washed three times with 0.3 ml of 0.1% Tween-20 in PBS. The non-specific interaction of unrelated proteins (e.g., serum peptides that may interfere with the analysis) with the antibody was reduced by incubating the pre-treated wells with a solution of 0.5% casein or 0.5%
20 gelatin in PBS for 30 min at 37°C. Wells were washed with 0.1% Tween-20 in PBS prior to analysis of media samples.

The conditioned media were diluted 1:20 in 0.95 ml of 0.1% CHAPS in PBS. Media samples (50 or 100 μ l/well) or α -sAPP standards (50 or 100 μ l/well) in a range of about 3 to 30 ng/ml were added to the wells for
25 a 60 min incubation at 37°C. The solution was then removed and each sample well was washed as described above. A horseradish peroxidase-conjugated goat affinity purified antibody (designated polycab #1) raised against human sAPP₆₉₅ was diluted in 0.1% Tween-20 in PBS plus 10% normal rabbit serum and added to the wells as the signal antibody. The
30 plates were incubated for 1-2 hr at 37°C and then washed to remove

-107-

unbound antibody. The chromogenic substrate K-Blue Solution (Elisa Technologies, Lexington KY) was added to the wells (0.1 ml/well) and allowed to incubate for 15 min at ambient temperature. The reactions were stopped by addition of 0.1 ml of a 9.8% solution of phosphoric acid. The optical density of the samples was measured by spectrophotometry at 450 nm. The concentration of α -sAPP in the media was estimated from the α -sAPP standard curve. Samples were analyzed in duplicate.

EXAMPLE 32

A METHOD DETECTING AN INDICATOR OR ALZHEIMER'S DISEASE

10 Total s-APP and α -sAPP levels in cerebrospinal fluid (CSF) of normal subjects and members of a Swedish family carrying mutations of the APP gene at codons 670 and 671 (APP_{670/671}) were measured and compared. The APP_{670/671} mutation in the Swedish family is associated with a high incidence of early onset Alzheimer's disease (AD). The clinical diagnosis of

15 AD in the Swedish family harboring the mutation was based on NINCDS-ADRDA criteria [McKhann, et al. (1984) Neurology 34:939-944]. The diagnosis was confirmed by neuropathologic examination of the brain of one deceased mutation carrier [Lannfelt, et al. (1994) Neurosci. Lett. 168:254-256]. Cognitive functioning was assessed with the Mini Mental State

20 Examination (MMSE) [Folstein, et al. (1975) J. Psychiatry Res. 12:189-198]. The presence or absence of the APP_{670/671} mutation was determined by polymerase chain reaction (PCR) nucleic acid amplification and restriction enzyme digestion according to a previously established procedure [Lannfelt, et al. (1993) Neurosci. Lett. 153:85-87].

25 Lumbar CSF was obtained from eight normal non-carriers in the family, two presymptomatic healthy mutation carriers, and four mutation carriers clinically symptomatic for AD. CSF samples were placed on ice, aliquoted and stored at -20°C until tested.

-108-

A. Measurement of APP Levels

Total sAPP and α -sAPP levels in the CSF samples were quantitated using a sandwich enzyme-linked immunosorbent assay (ELISA) and immunoblotting followed by laser-scanning densitometry, respectively.

- 5 Standards used in the assays were obtained by isolation of total sAPP and α -sAPP from medium conditioned by human neuroblastoma IMR32 cells [ATCC Accession No. CCL127] or the HGB695 cells, described above in Example 31B, as follows. Conditioned medium was filtered to remove large cell debris, and sAPP was extracted by passing the media over an anion
- 10 exchange column using Toyopearl DEAE 650C resin (Toso-Hass, Philadelphia, PA). The bound sAPP was eluted from the column using a linear gradient of 0 to 0.6 M NaCl in 50 mM sodium phosphate, pH 7.5. All sAPP-containing eluate fractions were pooled and loaded onto an immunoaffinity column containing either a monoclonal antibody that
- 15 specifically recognizes an amino-terminal epitope of human APP (for example, monoclonal antibody P2-1 raised against native human PN-2) [see, e.g., U.S. Patent No. 5,213,962] or goat polyclonal antibody polycab #1 (raised against sAPP) linked to Toyopearl AF-Tresyl 650 M resin (Toso-Hass). Bound sAPP was eluted from the column with 0.1 M sodium citrate,
- 20 pH 2.0. To separate α -sAPP from the other soluble forms of sAPP contained in total sAPP that do not contain at least the amino-terminal portion of A β , the total sAPP was loaded onto a Sepharose 4B immunoaffinity adsorption column containing a monoclonal antibody that recognizes an epitope within the first ~ 17 amino acids of A β (for example, monoclonal antibody 6E10).
- 25 Specifically bound α -sAPP was eluted from the column with 0.1 M sodium citrate, pH 3.0. The solution pH of the purified sAPPs was adjusted to 7.2 and 1-ml aliquots were stored at -70°C.

-109-

B. Quantitation of Total sAPP

The ELISA used to quantitate total sAPP levels in CSF samples employed either a monoclonal antibody, such as P2-1, discussed above, that specifically recognizes an amino-terminal epitope of human APP, or a polyclonal antibody, such as goat antibody polycab #1, also described above, as the capture antibody. The capture antibody was attached to the wells of a 96-well microtiter plate by incubating the plate with the antibody (that had been diluted in PBS, pH 7.2) for 60 min at 37°C. The plates were then washed three times with 0.3 ml of 0.1% Tween-20 in PBS. The wells were also incubated with a solution of 0.5% casein or 0.5% gelatin in PBS (150 or 200 µl/well) for 30 min. at 37 ° C.

CSF samples (50 or 100 µl diluted 1:20) or sAPP standards (50 or 100 µl) containing a range of 5 to 50 ng/ml were added to the wells and allowed to incubate for 60 min at 37°C. Following incubation, the wells were washed thoroughly with 0.1% Tween-20 in PBS. A goat anti-human APP polyclonal antibody raised against immunopurified APP from medium conditioned by cultured IMR32 human neuroblastoma cells (American Type Culture Collection Accession No. 127) conjugated to horseradish peroxidase was used as the signal antibody. The antibody was diluted 1:500 in PBS and 10% normal goat serum, pH 7.2, containing 0.1% Tween-20, added to the wells, and incubated for 60 min at 37 ° C. Unbound antibody was removed by washing as described above. To detect the bound antibody, 0.1 ml of the chromogenic substrate K-Blue Solution (Elisa Technologies, Lexington KY) was added to the wells and allowed to incubate for 15 min at ambient temperature. Reactions were stopped by addition of 0.1 ml of a 9.8% solution of phosphoric acid. The optical density of the samples was measured by spectrophotometry at 450 nm. The concentration of sAPP peptides in the CSF sample was estimated from the standard curve. Samples were analyzed in duplicate.

-110-

Total sAPP levels were also measured using quantitative immunoblotting essentially as described below for measurement of α -sAPP, except using a monoclonal antibody raised against a recombinant APP-containing fusion protein (e.g., 22C11 available from Boehringer Mannheim, Indianapolis, IN) at a concentration of 0.3 μ g/ml to specifically detect sAPP and using purified sAPP as a standard. Quantification of total sAPP by quantitative immunoblot gave a 95% correlation to quantification by ELISA.

C. Quantitation of α -sAPP

For immunoblot assays of α -sAPP contained in the CSF samples, 5-10 μ l of sample and purified standard α -sAPP of known concentrations were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Samples were diluted into Laemmli sample buffer and loaded onto 7.5% SDS-PAGE gel. After separation, the proteins were transferred to polyvinylidene difluoride membranes (PVDF Immobilon, Millipore, Bedford, MA) in CAPS transfer buffer (5 mM 3-[cyclohexylamine]-1-propanesulfonic acid, pH 11.0, 5% (v/v) methanol). Nonspecific binding of protein to membranes was blocked with PBS containing 5% (w/v) non-fat dried milk and then incubated for 1 hr with a monoclonal antibody (20ml of 0.2 μ g/ml) directed against the amino-terminus of A β (e.g., 6E10), and washed three times for one min each time in 20 ml of PBS and 0.1% Tween. Specifically bound antibody was detected using a biotinylated goat anti-mouse secondary antibody (Sigma) and a streptavidin-peroxidase conjugate (Amersham, Arlington Heights, IL) in combination with an enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL). The blots were exposed to Kodak Scientific Imaging film X-OMAT AR and developed using a Kodak X-OMAT developer. Quantitation of the α -sAPP protein in the blots was performed by laser-scanning densitometry. Developed films within the linear range (or multiple exposures) were scanned at 50 μ M pixel size using a densitometer (Molecular Dynamics, Sunnyvale, CA), and the data were quantified using

-111-

the ImageQuaNT software system (Molecular Dynamics). Quantified volumes of α -sAPP standard were used to generate standard curves. From the standard curves, the levels of α -sAPP in ng/ml were determined.

5 D. Comparison of sAPP and α -sAPP Levels in CSF of Normal Subjects and Mutation Carriers

Assays of sAPP and α -sAPP levels in CSF from normal subjects and Swedish mutation carriers were performed. Mann-Whitney non-parametric statistics were used for comparison of the data from the experimental groups. Correlations were investigated with Pearson's and Spearman's rank
10 correlation coefficients. Significance levels were set at $p < 0.05$. The CSF of diseased carriers had lower levels of α -sAPP than the CSF samples of non-carriers, with no overlap between the two groups ($z = -2.72$; $p = 0.007$). The CSF obtained from the four AD subjects had lower levels of α -sAPP than that of the two pre-symptomatic AD carriers. There was a strong
15 inverse correlation between α -sAPP concentration and age in the mutation carriers ($R = 0.94$; $p = 0.005$). In the mutation carriers, ~25% of the total sAPP in CSF was α -sAPP compared to 33% in CSF of non-carriers. This was a statistically significant difference.

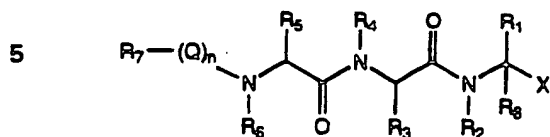
The results indicate that α -sAPP and the ratio of α -sAPP to total sAPP
20 in CSF are useful markers in the detection of neurodegenerative disorders characterized by cerebral deposition of amyloid (e.g., AD) and in monitoring the progression of such disease. Furthermore, this assay system can be used in monitoring therapeutic intervention of these diseases.

Since modifications will be apparent to those of skill in this art, it is
25 intended that this invention be limited only by the scope of the appended claims.

-112-

WE CLAIM:

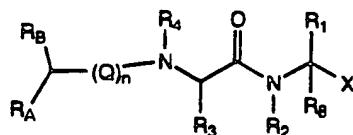
1. A compound of the formula (I) or (II):



10

I

15



20

II

25 or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof, with the proviso that: (A) when the compounds have formula (I): (1) at least one of R_1 , R_3 and R_5 is not a side chain of a naturally-occurring amino acid; and (2) when X is an aldehyde, the side chain of the non-naturally occurring amino acid is other than the side chain of norleucine or norvaline, and when the compounds have formula (II) and X is an aldehyde, R_1 cannot be the side chain of norleucine or norvaline, wherein:

30

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi) or (vii) as follows:

- 35 (i) R_1 , R_3 , R_5 , and R_8 , are each independently selected from a side chain of a naturally occurring α -amino acid, H, alkyl, preferably lower (C_{1-6}) alkyl, alkenyl, preferably C_{2-10} alkenyl, alkynyl, preferably

-113-

C₂₋₆ alkynyl, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, Y-substituted aryl, aralkyl, aralkenyl, aralkynyl, and Z-substituted heteroaryl, heteroaralkyl, heteroaralkenyl, in which Y is selected from halogen, lower alkyl, alkoxy, OH, haloalkyl, preferably CF₃, NO₂, nitrile, S-alkyl, phenyl, and -NRR', R and R' are independently selected from H or alkyl, preferably lower alkyl, OH and halo-lower alkyl, particularly CF₃. Z is lower alkyl, preferably C₁₋₄ alkyl, or halo lower alkyl, preferably C₁₋₄ haloalkyl, more preferably CF₃;

R₂, R₄, R₆, and R₈ are each independently selected from among H and lower alkyl, preferably C₁₋₄ alkyl;

R₇ is selected from among C₁₋₆ alkyl, aryl, alkenyl, 9-fluorenyl, aralkyl, aralkenyl, aralkynyl the aryl groups are unsubstituted or are substituted with Z;

Q is selected from among -C(O)-, -O-C(O)-, -C(O)O-, -S(O)₂- and HN-C(O)-;

n is zero or one;

R_A is -(T)_m-(D)_m-R₁ in which T is O or NH or carbon, and D is C₁₋₄ alkyl or C₂₋₄ alkene; and m is zero or one;

X is selected from -(CH₂)_rC(O)H, -(CH₂)_rC(O)haloalkyl, -(CH₂)_rC(O)(CH₂)_rCHN₂, -C(CH₂)_r(O)C(CH₂)_r(O)OR_D, -(CH₂)_rC(O)(CH₂)_rC(O)NR_DR_E, -(CH₂)_rC≡N, -(CH₂)_rC(OH)(CH₂)_rC(O)U, -(CH₂)_rC(OH)CH₂C(O)U, -(CH₂)_rC(O)W and -(CH₂)_rC(O)CH₂W, in which: R_D and R_E are independently selected from among H, alkyl, preferably lower alkyl, more preferably C₁₋₄ alkyl, phenyl, benzyl, and phenethyl; U is -OR_D or -NR_DR_E, and W is -OR_D, -SR_D, and -NR_DR_E, or heterocyclic moiety, preferably containing 4-6, more preferably 5 or 6 members in the ring, and preferably one or two heteroatoms, selected from O, S, or N, in the ring, and r is 0-5, preferably 0-3, more preferably 0 or 1, most preferably 0; or

-114-

- (ii) $R_1, R_2, R_3, R_4, R_5, R_6$, and X are selected as in (i),
(iv)(a-c) with R_6 being H or (v)(a-c);

V is OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH;

n is zero; and

R_6 and R_7 are selected so that with the atoms to which each is attached they form a heterocyclic moiety, which:

(a) contains from 3 to 21 members and one or two or more fused rings, each ring containing preferably 3 to 7, more preferably 4 to 6, members, and is preferably morpholino, thiomorpholino, pyrrolidinyl, V -substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl, or a reduced isoquinoline, preferably 1,2,3,4-tetrahydroisoquinoline;

(b) does not contain adjacent heteroatoms;

(c) is unsubstituted or substituted with one or more substituents selected from Y , more preferably from V , and most preferably selected from among OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH; or

- (iii) $R_1, R_2, R_3, R_4, R_5, R_6$, and X are selected as in (i);

V is as defined in (ii);

Q is $C(O)$;

n is one; and

R_6 and R_7 are each independently selected as follows:

(a) from carbonyl ($C=O$), phenyl, a heteroatom, lower alkyl, preferably C_{1-3} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-3} alkyl, and

(b) each is unsubstituted or substituted with Y , preferably with V , and

-115-

(c) together with the atoms to which they are attached form a cyclic moiety, preferably a 4-6 membered cyclic or 8-12 membered bicyclic moiety, and

(d) R_6 and R_7 are selected with the proviso that when two or more heteroatoms are present there is a carbon atom between the heteroatoms; and

(e) the cyclic moiety is preferably succinimide, phthalimide or maleimide; or

(iv) R_3 , R_4 , R_5 , R_6 , R_7 , R_A , R_B , Q , X and n are as defined in any of (i)-(iii) or (v)-(vii),

V is as defined in (ii);

R_8 is H; and

R_1 and R_2 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_1 and R_2 are unsubstituted or substituted with Y , preferably with V , and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V -substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

(v) R_1 , R_2 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are as defined in any of (i)-(iv) or (vi)-(vii);

V is as defined in (ii);

R_3 and R_4 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the

-116-

proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) is unsubstituted or substituted with Y, preferably with V, and

5

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl;

10

(vi) $R_1, R_2, R_3, R_4, R_7, R_8, Q, X$ and n are as defined in any of (i), (iv)(a-c) with R_8 being H or (v)(a-c);
V is as defined in (ii);

R_5 and R_6 are each independently selected as follows:

15

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_5 and R_6 are unsubstituted or substituted with Y, preferably with V, and

20

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

25

(vii) $R_1, R_2, R_3, R_4, R_6, R_8$, and X are selected as in (i), (iv)(a-c) with R_8 being H or (v)(a-c);
V is as defined in (ii);

n is zero; and

R_5 and R_7 are each independently selected as follows:

-117-

(a) from lower alkyl, preferably C₁₋₄ alkyl, or lower alkyl linked to a heteroatom, preferably C₁₋₄ alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

5 (b) R₅ and R₇ are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl.

10

2. The compounds of claim 1, wherein at least one of R₂, R₄, R₆, and R₈ are N-methylated.

3. A compound of claim 1 or claim 2, wherein:

15 R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R_A, R_B, X, Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi), or (vii) as follows:

(i) R₁ is a straight or branched chain carbon chain containing 2 to 6 carbons and one unsaturated a double bond, or is cyclic moiety containing from 5 to 6 members;

20 R₂, R₄, and R₆ are each independently selected from methyl or ethyl or propyl;

R₃ is selected from among C₁₋₄ alkyl, phenyl, naphthyl, benzyl, hydroxyphenyl, 1-aminobutyl, acetamide and *iso*-butyl;

R₅ is C₁₋₄ alkyl;

25 R₆ is H or C₁₋₄ alkyl;

R₇ - (Q)_n is selected from among acetyl, benzyloxycarbonyl (Cbz), 9-fluorenylmethylcarbamate (Fmoc), Ac, Boc and tosyl;

Q is -C(O)-, -S(O)₂- or -O-C(O);

R₈ is C₁₋₄ alkyl or C₂₋₄ alkenyl;

-118-

$R_A = -(T)_n-(D)_n-R_1$, in which T is oxygen, or nitrogen, and D is C_{1-4} alkyl or C_{2-4} alkenyl; and

X is selected from among aldehyde, α -ketoester, α -ketoamide, trifluoromethylketone, diazomethylketone and nitrile; or

- 5 (ii) $R_1, R_2, R_3, R_4, R_5, R_6, R_A, X, Y$ and R_B are selected as in (i), (iv) or (v);

V is OH or halogen;

n is zero; and

- 10 R_6 and R_7 together with the atoms to which each is attached form a moiety selected from among morpholino, thiomorpholino, pyrrolidinyl, 4-hydroxy pyrrolidinyl, and 1,2,3,4-tetrahydroisoquinoline; or

- (iii) $R_1, R_2, R_3, R_4, R_5, R_6, R_A, X$ and R_B are selected as in (i);

V is as defined in (ii);

- 15 Q is C(O);

n is one; and

R_6 and R_7 together with the atoms to which each is attached form succinimide, phthalimide or maleimide; or

- 20 (iv) $R_3, R_4, R_5, R_6, R_7, R_A, R_B, Q, X$ and n are as defined in any of (i)-(iii) or (v)-(vii),

V is as defined in (ii);

R_B is H; and

- 25 R_1 and R_2 together with the atoms to which each is attached form a moiety selected from among morpholino, thiomorpholino, pyrrolidinyl, and 4-hydroxy pyrrolidinyl; or

- (v) $R_1, R_2, R_3, R_4, R_5, R_6, R_A, R_B, X, Q$ and n are as defined in any of (i)-(iv) or (vi)-(vii);

V is as defined in (ii);

-119-

R_3 and R_4 together with the atoms to which each is attached form a moiety selected from among morpholino, thiomorpholino, pyrrolidinyl, and 4-hydroxy pyrrolidinyl; or

(vi) $R_1, R_2, R_3, R_4, R_7, R_8, R_A, R_B, Q, X$ and n are as defined in any of (i), (iv) or (v);

V is as defined in (ii);

R_5 and R_6 together with the atoms to which each is attached form a moiety selected from among morpholino, thiomorpholino, pyrrolidinyl, and 4-hydroxy pyrrolidinyl; or

(vii) $R_1, R_2, R_3, R_4, R_6, R_8, R_A, X$ and R_B are selected as in (i) (iv) or (v);

V is as defined in (ii);

n is zero; and

R_5 and R_7 together with the atoms to which each is attached form a moiety selected from among morpholino, thiomorpholino, pyrrolidinyl, and 4-hydroxy pyrrolidinyl.

4. A compound of any of claims 1-3, wherein at least one of R_1, R_3 , and R_6 are selected from the group consisting of C_{2-6} alkenyl and C_{2-6} alkynyl.

5. A compound of any of claims 1-3, wherein at least R_1 and R_2, R_3 and R_4, R_5 and R_7 , and/or R_6 and R_7 together with the atoms to which each is attached form 4-hydroxy pyrrolidinyl.

6. A compound of any of claims 1-5, wherein:

R_8 is H; and

the heterocyclic ring moiety containing R_1 and R_2 and the atoms to which they are attached, is selected from among morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl.

-120-

7. A compound of any of claims 1-6, wherein:
(Q)_n is a carbonyl group; and
the heterocyclic ring moiety containing R₆ and R₇ and the atoms
to which they are attached is selected from among succinimide, phthalimide
5 or maleimide, and is preferably phthalimide.
8. A compound of any of claims 1-6, wherein:
n is zero; and
the heterocyclic ring moiety containing R₆ and R₇ and the atoms
to which they are attached is morpholino, thiomorpholino, pyrrolidinyl or V-
10 substituted pyrrolidinyl.
9. A compound of any of claims 1-6, wherein the heterocyclic ring
moiety containing R₆ and R₇ is 4-hydroxy pyrrolidinyl or 1,2,3,4-
tetrahydroisoquinoline.
10. A compound of any of claims 1-6, wherein:
15 n is zero; and
R₃ and R₄ or R₅ and R₇ are taken together with the atoms to
which they are attached form morpholino, thiomorpholino, pyrrolidinyl, or V-
substituted pyrrolidinyl, preferably 4-hydroxy pyrrolidinyl.
11. A compound of any of claims 1-6, wherein:
20 at least one of R₁, R₃, and R₅ are selected from among of 2-
methyl-propene, 2-butene, cyclohexylglycine and cyclohexylalanine.
12. A compound of any of claims 1-11, wherein X is selected from
among an aldehyde, α -ketoester, α -ketoamide, trifluoromethylketone,
diazomethylketone, or nitrile.
13. A compound of any of claims 1-11, wherein X is selected from
25 among an aldehyde, α -ketoester or α -ketoamide.
14. A compound of any of claims 1-11, wherein at least one of R₁,
R₃, and R₅ is 2-methyl-propene, 2-butene, cyclohexyl or cyclohexylmethyl.
15. A compound of any of claims 1-11, wherein at least one of R₁,
30 R₃, and R₅ is selected from among 2-methyl-propene, 2-butene,

-121-

cyclohexylglycine, and cyclohexylalanine, and X is selected from among C(O)H, C(O)-C(O)OR_D, C(O)-C(O)-NR_DR_E, C(O)CH₂N₂, C(O)CF₃, -(CH₂)₂C≡N, and C≡N.

16. A compound of any of claims 1-11 and 14, wherein X is selected from among C(O)H, C(O)-C(O)OR_D, C(O)-C(O)-NR_DR_E, C(O)CH₂N₂, C(O)CF₃, and C≡N.

17. A compound of any of claims 1-16, wherein X is selected from among C(O)H, C(O)-C(O)OR_D, C(O)-C(O)-NR_DR_E, C(O)CH₂CHN₂, C(O)CF₃ and C≡N.

18. A compound of claim 1, wherein R_B, R_A with the atom to which each is attached and (Q)_n form (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl] or (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl or valeroyl].

19. A compound of claim 1 or claim 2, wherein:

R₁ is 2-methyl propene, 2-butene, cyclohexyl or cyclohexylmethyl;

R₂, R₄, and R₈ are each independently methyl or ethyl;

R₃ is *iso*-butyl or phenyl;

R₅ is *iso*-butyl;

R₆ is H or methyl;

R₇ - (Q)_n are acetyl or benzyloxycarbonyl (Cbz);

Q is -C(O)- or -O-C(O)

R₉ is *iso*-butyl;

R_A = -(T)_m-(D)_m-R₁, in which T is O or NH, and D is C₃₋₄ alkenyl; and

X is aldehyde, α -ketoester or α -ketoamide.

20. The compounds of any of claims 1-13 and 15-19, wherein R₁ is 2-methyl propene or 2-butene.

21. The compounds of any of claims 1-20 that have formula (I).

22. The compounds of any of claims 1-20 that have formula (II).

23. The compounds of claim 1, wherein X is -(CH₂)₂C≡N, and C≡N.

24. A compound of claim 1 selected from among *N*-Ac-L-Leu-L-Leu-DL-cyclohexylalaninal; (2*SR*)-*N*-Cbz-L-Lu-L-Leu *N*-[2-(4-methyl-4-

-122-

pentenal))amide; (2*SR*)-H-L-Leu *N*-[2-(ethyl 4-methyl-4-pentenoate))amide hydrochloride; (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl]-L-Leu *N*-[2-(4-methyl-4-pentenal))amide; (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu-*N*-[2-(4-methyl-4-pentenal))amide; *N*-Cbz-L-Leu-L-Leu-DL-cyclohexylglycinal; *N*-Ac-L-Leu-L-Leu-DL-cyclohexylglycinal; (2*SR*)-*N*-Ac-L-Leu-L-Leu *N*-[2-(*trans*-4-hexenal))amide; (2*SR*)-*N*-Ac-L-Leu-L-Leu *N*-[2-(4-methyl-4-pentenal))amide; (2*SR*)-*N*-Cbz-L-Leu-L-Phe-DL-cyclohexylalaninal; and (2*SR*)-*N*-Cbz-L-Leu-L-Phe-*N*-[2-(4-methyl-4-pentenal))amide/*N*-Ac-L-Leu-L-Leu-L-Nle-COCHN₂; (2*S*)-*N*-Ac-L-Leu-L-Leu *N*-[2-(pentanenitrile))amide; (2*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[2-(4-methyl-4-pentenal))amide; (2*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu-*N*-[2-(4-methyl-4-pentenal))amide; (2*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[2-(*trans*-4-hexenal))amide; and (2*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu-*N*-[2-(*trans*-4-hexenal))amide.

25. A compound that is an α -keto ester or α -keto amides of any of the compounds of any of claims 1-24.

26. A compound of claim 25 selected from among (3*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenoate)) amide, (3*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenoate)) amide, (3*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-hexenoate)) amide, (3*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[3-(methyl 2-keto-5-methyl-3-hexenoate))amide, (3*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[3-(ethyl 2-keto-5-methyl-3-hexenoate))amide, (3*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[3-(benzyl 2-keto-5-methyl-3-hexenoate))amide, (3*SR*)-*N*-Cbz-L-Leu-L-Leu-*N*-[3-(methyl 2-keto-5-methyl-3-hexenamide))amide, (3*SR*)-*N*-Cbz-L-Leu-L-Leu-*N*-[3-(ethyl 2-keto-5-methyl-3-hexenamide))amide, (3*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-hexenamide)) amide, (3*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[3-(methyl 2-keto-5-methyl-3-hexenamide))amide, (3*SR*)-*N*-Cbz-L-Pro-L-Leu-*N*-[3-(ethyl 2-keto-5-methyl-3-hexenamide))amide, (3*SR*)-*N*-Cbz-L-Pro-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-hexenamide)) amide, (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenoate)) amide, (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-

- hexenoate)) amide, (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-hexenoate)) amide, (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenamamide)) amide, (3*SR*)-*N*-Cbz-L-HydroxyPro-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenamamide)) amide, (3*SR*)-*N*-Cbz-L-HydroxyPro-
- 5 L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-hexenamamide)) amide, (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenoate)) amide, (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenoate)) amide, (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[3-
- 10 (benzyl 2-keto-5-methyl-3-hexenoate)) amide, (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenamamide)) amide, (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenamamide)) amide, (2*SR*)-*N*-[(2*R*)-[2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu *N*-[3-
- 15 (benzyl 2-keto-5-methyl-3-hexenamamide)) amide, (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl]-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenoate)) amide, (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl]-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenoate)) amide, (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl]-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-hexenoate)) amide, (2*SR*)-*N*-[(2*S*)-2-
- 20 benzoxo-4-methylpentanoyl]-L-Leu *N*-[3-(methyl 2-keto-5-methyl-3-hexenamamide)) amide, (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl]-L-Leu *N*-[3-(ethyl 2-keto-5-methyl-3-hexenamamide)) amide, and (2*SR*)-*N*-[(2*S*)-2-benzoxo-4-methylpentanoyl]-L-Leu *N*-[3-(benzyl 2-keto-5-methyl-3-hexenamamide)) amide.
- 25 27. A compound of claim 1, selected from among *N*-Ac-L-Leu-L-L u-DL-cyclohexylalaninal, *N*-Cbz-L-Leu-L-L u-DL-cyclohexylglycinal, (2*SR*)-*N*-Ac-L-Leu-L-Leu *N* [2-(*trans*-4-hexenal)) amide.
28. A compound of claim 1, selected from among (2*SR*)-*N*-Cbz-L-Leu-L-Leu *N*-[2-(4-methyl-4-pentenol)) amide, (2*SR*)-*N*-[(2*S*)-2-benz oxo-4-
- 30 methylpentanoyl]-L-L u-[2-(4-methyl-4-pentenol)) amide, (2*SR*)-*N*-[(2*R*)-[2-

-124-

(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu-N-[2-(4-methyl-4-pentenal)]amide, (2*SR*)-N-Cbz-L-Leu-L-Phe-DL-cyclohexylalaninal, and (2*SR*)-N-Cbz-L-Leu-L-Phe-N-[2-(4-methyl-4-pentenal)]amide.

29. A compound of claim 1 that is (2*SR*)-N-Cbz-L-Leu-L-Leu N-[2-(4-methyl-4-pentenal)]amide or (2*SR*)-N-Cbz-L-Leu-L-Phe-DL-cyclohexylalaninal.

30. A compound of claim 1 that is (2*SR*)-N-[(2*S*)-2-benzyloxy-4-methylpentanoyl]-L-Leu N-[2-(4-methyl-4-pentenal)]amide or (2*SR*)-N-[(2*R*)-2-(1'-phenyl-1'-propene)-4-methylpentanoyl]]-L-Leu-N-[2-(4-methyl-4-pentenal)]amide.

31. A compound of claim 2, wherein:

R_1 is 2-methyl propene, 2-butene, cyclohexyl or cyclohexylmethyl;

R_2 , R_4 , and R_8 are each independently methyl, ethyl or hydrogen;

R_3 is *iso*-butyl, benzyl or phenyl;

15 R_5 is *iso*-butyl;

R_6 is H or methyl;

$R_7 - (Q)_n$ are acetyl or benzyloxycarbonyl (Cbz);

Q is -C(O)- or -O-C(O)

R_8 is *iso*-butyl;

20 $R_A = -(T)_m-(D)_m-R_1$, in which T is oxygen, NH or carbon, and D is C_{3-4} alkenyl; and

X is aldehyde, α -ketoester or α -ketoamide.

32. A compound of claim 2, wherein:

R_1 is 2-methyl propene, 2-butene, cyclohexyl or cyclohexylmethyl;

25 R_2 , R_4 , and R_8 are hydrogen;

R_3 is *iso*-butyl, benzyl or phenyl;

R_5 is *iso*-butyl;

R_6 is H or methyl;

$R_7 - (Q)_n$ are acetyl or benzyloxycarbonyl (Cbz);

30 Q is -C(O)- or -O-C(O)

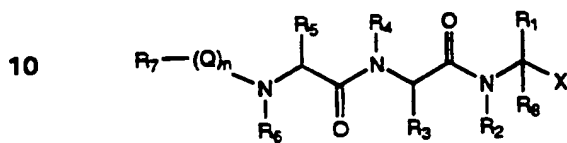
-125-

R_8 is *iso*-butyl;

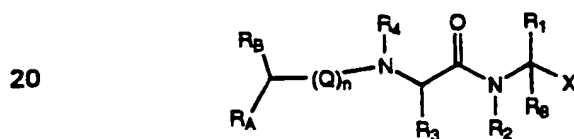
$R_A = -(T)_m-(D)_n-R_1$, in which T is oxygen, NH or carbon, and D is C_{3-4} alkenyl; and

X is aldehyde, α -ketoester or α -ketoamide.

- 5 33. A method of modulating the processing of amyloid precursor protein (APP), comprising contacting the APP with a compound of formula (I) or (II):



15 I



25 II

- 30 or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof, wherein:

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X, Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi) or (vii) as follows:

- 35 (i) R_1 , R_3 , R_5 , and R_8 , are each independently selected from a side chain of a naturally occurring α -amino acid, H, alkyl, preferably lower (C_{1-6}) alkyl, alkenyl, preferably C_{2-10} alkenyl, alkynyl, preferably C_{2-6} alkynyl, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, Y-substituted aryl, aralkyl, aralkenyl,

-126-

aralkynyl, and Z-substituted heteroaryl, heteroaralkyl, heteroaralkenyl, in which Y is selected from halogen, lower alkyl, alkoxy, OH, haloalkyl, preferably CF₃, NO₂, nitrile, S-alkyl, phenyl, and -NRR', R and R' are independently selected from H or alkyl, preferably lower alkyl, OH and halo-lower alkyl, particularly CF₃, Z is lower alkyl, preferably C₁₋₄ alkyl, or halo lower alkyl, preferably C₁₋₄ haloalkyl, more preferably CF₃;

R₂, R₄, R₆, and R₈ are each independently selected from among H and lower alkyl, preferably C₁₋₄ alkyl;

R₇ is selected from among C₁₋₆ alkyl, aryl, alkenyl, 9-fluorenyl, aralkyl, aralkenyl, aralkynyl the aryl groups are unsubstituted or are substituted with Z;

Q is selected from among -C(O)-, -O-C(O)-, -C(O)O-, -S(O)₂- and HN-C(O)-;

n is zero or one;

R_A is -(T)_m-(D)_m-R₁ in which T is O or NH, and D is C₁₋₄ alkyl or C₂₋₄ alkene; and m is zero or one;

X is selected from -(CH₂)_rC(O)H, -(CH₂)_rC(O)haloalkyl, -(CH₂)_rC(O)(CH₂)_rCHN₂, -C(CH₂)_r(O)C(CH₂)_r(O)OR_D, -(CH₂)_rC(O)(CH₂)_rC(O)NR_DR_E, -(CH₂)_rC≡N, -(CH₂)_rC(OH)(CH₂)_rC(O)U, -(CH₂)_rC(OH)CH₂C(O)U, -(CH₂)_rC(O)W and -(CH₂)_rC(O)CH₂W, in which: R_D and R_E are independently selected from among H, alkyl, preferably lower alkyl, more preferably C₁₋₄ alkyl, phenyl, benzyl, and phenethyl; U is -OR_D or -NR_DR_E, and W is -OR_D, -SR_D, and -NR_DR_E, or heterocyclic moiety, preferably containing 4-6, more preferably 5 or 6 members in the ring, and preferably one or two heteroatoms, selected from O, S, or N, in the ring, and r is 0-5, preferably 0-3, more preferably 0 or 1, most preferably 0; or

-127-

- (ii) $R_1, R_2, R_3, R_4, R_5, R_6$, and X are selected as in (i),
(iv)(a-c) with R_6 being H or (v)(a-c);

V is OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH;

n is zero; and

R_6 and R_7 are selected so that with the atoms to which each is attached they form a heterocyclic moiety, which:

(a) contains from 3 to 21 members and one or two or more fused rings, each ring containing preferably 3 to 7, more preferably 4 to 6, members, and is preferably morpholino, thiomorpholino, pyrrolidinyl, V -substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl, or a reduced isoquinoline, preferably 1,2,3,4-tetrahydroisoquinoline;

(b) does not contain adjacent heteroatoms;

(c) is unsubstituted or substituted with one or more substituents selected from Y , more preferably from V , and most preferably selected from among OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH; or

- (iii) $R_1, R_2, R_3, R_4, R_5, R_6$, and X are selected as in (i);

V is as defined in (ii);

Q is $C(O)$;

n is one; and

R_6 and R_7 are each independently selected as follows:

(a) from carbonyl ($C=O$), phenyl, a heteroatom, lower alkyl, preferably $C_{1,3}$ alkyl, or lower alkyl linked to a heteroatom, preferably $C_{1,3}$ alkyl, and

(b) each is unsubstituted or substituted with Y , preferably with V , and

-128-

(c) together with the atoms to which they are attached form a cyclic moiety, preferably a 4-6 membered cyclic or 8-12 membered bicyclic moiety, and

(d) R_6 and R_7 are selected with the proviso that when two or more heteroatoms are present there is a carbon atom between the heteroatoms; and

(e) the cyclic moiety is preferably succinimide, phthalimide or maleimide; or

(iv) R_3 , R_4 , R_5 , R_6 , R_7 , R_A , R_B , Q, X and n are as defined in any of (i)-(iii) or (v)-(vii),

V is as defined in (ii);

R_8 is H; and

R_1 and R_2 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_1 and R_2 are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

(v) R_1 , R_2 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X, Q and n are as defined in any of (i)-(iv) or (vi)-(vii);

V is as defined in (ii);

R_3 and R_4 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the

-129-

proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) is unsubstituted or substituted with Y, preferably with V, and

5 (c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl;

10 (vi) $R_1, R_2, R_3, R_4, R_7, R_8, Q, X$ and n are as defined in any of (i), (iv)(a-c) with R_6 being H or (v)(a-c);

V is as defined in (ii);

R_5 and R_6 are each independently selected as follows:

15 (a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_5 and R_6 are unsubstituted or substituted with Y, preferably with V, and

20 (c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

25 (vii) $R_1, R_2, R_3, R_4, R_6, R_8$, and X are selected as in (i),

(iv)(a-c) with R_5 being H or (v)(a-c);

V is as defined in (ii);

n is zero; and

R_5 and R_7 are each independently selected as follows:

-130-

(a) from lower alkyl, preferably C₁₋₄ alkyl, or lower alkyl linked to a heteroatom, preferably C₁₋₄ alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

5 (b) R₅ and R₇ are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl.

10

34. A method of modulating the processing of amyloid precursor protein (APP), comprising contacting the APP with a compound of any of claims 1-33.

15 35. A method of treating a neurodegenerative disease that is characterized by the deposition of cerebral amyloid, comprising administering to a patient a therapeutically effective amount of a compound of formula (I) or (II):

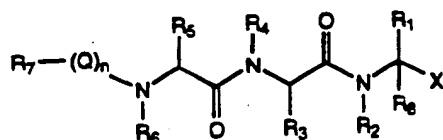
20

25

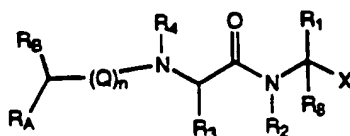
30

35

-131-



I



II

or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof, wherein:

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi) or (vii) as follows:

- 5 (i) R_1 , R_3 , R_5 , and R_8 , are each independently selected from a side chain of a naturally occurring α -amino acid, H, alkyl, preferably lower (C_{1-6}) alkyl, alkenyl, preferably C_{2-10} alkenyl, alkynyl, preferably C_{2-6} alkynyl, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, Y-substituted aryl, aralkyl, aralkenyl, aralkynyl, and Z-substituted heteroaryl, heteroaralkyl, heteroaralkenyl,
- 10 in which Y is selected from halogen, lower alkyl, alkoxy, OH, haloalkyl, preferably CF_3 , NO_2 , nitrile, S-alkyl, phenyl, and $-NRR'$, R and R' are independently selected from H or alkyl, preferably lower alkyl, OH and halo-lower alkyl, particularly CF_3 , Z is lower alkyl,
- 15 preferably C_{1-4} alkyl, or halo lower alkyl, preferably C_{1-4} haloalkyl, more preferably CF_3 ;

-132-

R_2 , R_4 , R_6 , and R_8 are each independently selected from among H and lower alkyl, preferably C_{1-4} alkyl;

R_7 is selected from among C_{1-6} alkyl, aryl, alkenyl, 9-fluorenyl, aralkyl, aralkenyl, aralkynyl the aryl groups are unsubstituted or are substituted with Z;

Q is selected from among $-C(O)-$, $-O-C(O)-$, $-C(O)O-$, $-S(O)_2-$ and $HN-C(O)-$;

n is zero or one;

R_A is $-(T)_m-(D)_m-R_1$ in which T is O or NH, and D is C_{1-4} alkyl or C_{2-4} alkene; and m is zero or one;

X is selected from $-(CH_2)_rC(O)H$, $-(CH_2)_rC(O)haloalkyl$, $-(CH_2)_rC(O)(CH_2)_rCHN_2$, $-C(CH_2)_r(O)C(CH_2)_r(O)OR_D$, $-(CH_2)_rC(O)(CH_2)_rC(O)NR_DR_E$, $-(CH_2)_rC\equiv N$, $-(CH_2)_rC(OH)(CH_2)_rC(O)U$, $-(CH_2)_rC(OH)CH_2C(O)U$, $-(CH_2)_rC(O)W$ and $-(CH_2)_rC(O)CH_2W$, in which:

R_D and R_E are independently selected from among H, alkyl, preferably lower alkyl, more preferably C_{1-4} alkyl, phenyl, benzyl, and phenethyl; U is $-OR_D$ or $-NR_DR_E$, and W is $-OR_D$, $-SR_D$, and $-NR_DR_E$, or heterocyclic moiety, preferably containing 4-6, more preferably 5 or 6 members in the ring, and preferably one or two heteroatoms, selected from O, S, or N, in the ring, and r is 0-5, preferably 0-3, more preferably 0 or 1, most preferably 0; or

(ii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i),

(iv)(a-c) with R_8 being H or (v)(a-c);

V is OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH;

n is zero; and

R_6 and R_7 are selected so that with the atoms to which each is attached they form a heterocyclic moiety, which:

-133-

5 (a) contains from 3 to 21 members and one or two or more fused rings, each ring containing preferably 3 to 7, more preferably 4 to 6, members, and is preferably morpholino, thiomorpholino, pyrrolidinyl, V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl, or a reduced isoquinoline, preferably 1,2,3,4,tetrahydroisoquinoline;

(b) does not contain adjacent heteroatoms;

10 (c) is unsubstituted or substituted with one or more substituents selected from Y, more preferably from V, and most preferably selected from among OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH; or

(iii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i);

V is as defined in (ii);

15 Q is C(O);

n is one; and

R_6 and R_7 are each independently selected as follows:

20 (a) from carbonyl (C=O), phenyl, a heteroatom, lower alkyl, preferably C_{1-3} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-3} alkyl, and

(b) each is unsubstituted or substituted with Y, preferably with V, and

25 (c) together with the atoms to which they are attached form a cyclic moiety, preferably a 4-6 membered cyclic or 8-12 membered bicyclic moiety, and

(d) R_6 and R_7 are selected with the proviso that when two or more heteroatoms are present there is a carbon atom between the heteroatoms; and

30 (e) the cyclic moiety is preferably succinimide, phthalimide or maleimide; r

-134-

(iv) R_3 , R_4 , R_5 , R_6 , R_7 , R_A , R_B , Q , X and n are as defined in any of (i)-(iii) or (v)-(vii),

V is as defined in (ii);

R_8 is H; and

5 R_1 and R_2 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

10 (b) R_1 and R_2 are unsubstituted or substituted with Y , preferably with V , and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V -substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

15

(v) R_1 , R_2 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are as defined in any of (i)-(iv) or (vi)-(vii);

V is as defined in (ii);

20 R_3 and R_4 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

25 (b) is unsubstituted or substituted with Y , preferably with V , and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpho-

-135-

lino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl;

(vi) $R_1, R_2, R_3, R_4, R_7, R_8, Q, X$ and n are as defined in any of (i), (iv)(a-c) with R_8 being H or (v)(a-c);

5 V is as defined in (ii);

R_5 and R_6 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

10

(b) R_5 and R_6 are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

15

(vii) $R_1, R_2, R_3, R_4, R_6, R_8$, and X are selected as in (i),

(iv)(a-c) with R_8 being H or (v)(a-c);

20 V is as defined in (ii);

n is zero; and

R_5 and R_7 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

25

(b) R_5 and R_7 are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered

30

-136-

heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl.

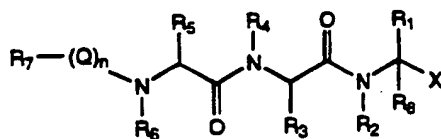
36. The method of claim 35, wherein the disease is selected from the group consisting of Alzheimer's disease, cognition deficits, Downs Syndrome, cerebral hemorrhage with amyloidosis, dementia pugilistica, and head trauma.

37. The method of claim 35, wherein the disease is Alzheimer's disease.

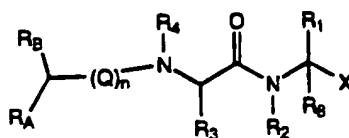
38. A method of treating a neurodegenerative disease that is characterized by the deposition of cerebral amyloid, comprising administering to a patient a therapeutically effective amount of a compound of any of claims 1-32.

39. A method of treating a patient suffering from a disease characterized by a degradation of the neuronal cytoskeleton resulting from a thrombolytic or hemorrhagic stroke, comprising administering to the patient a therapeutically effective amount of a compound of formula (I) or (II):

-137-



I



II

or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof, wherein:

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi) or (vii) as follows:

- (i) R_1 , R_3 , R_5 , and R_8 , are each independently selected from a side chain of a naturally occurring α -amino acid, H, alkyl, preferably lower (C_{1-6}) alkyl, alkenyl, preferably C_{2-10} alkenyl, alkynyl, preferably C_{2-6} alkynyl, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, Y-substituted aryl, aralkyl, aralkenyl, aralkynyl, and Z-substituted heteroaryl, heteroaralkyl, heteroaralkenyl, in which Y is selected from halogen, lower alkyl, alkoxy, OH, haloalkyl, preferably CF_3 , NO_2 , nitrile, S-alkyl, phenyl, and $-\text{NRR}'$, R and R' are independently selected from H or alkyl, preferably lower alkyl, OH and halo-lower alkyl, particularly CF_3 . Z is lower alkyl, preferably C_{1-4} alkyl, or halo lower alkyl, preferably C_{1-4} haloalkyl, or preferably CF_3 ;

SUBSTITUTE SHEET (RULE 26)

-138-

R_2 , R_4 , R_6 , and R_8 are each independently selected from among H and lower alkyl, preferably C_{1-4} alkyl;

R_7 is selected from among C_{1-6} alkyl, aryl, alkenyl, 9-fluorenyl, aralkyl, aralkenyl, aralkynyl the aryl groups are unsubstituted or are substituted with Z;

Q is selected from among $-C(O)-$, $-O-C(O)-$, $-C(O)O-$, $-S(O)_2-$ and $HN-C(O)-$;

n is zero or one;

R_A is $-(T)_m-(D)_m-R_1$ in which T is O or NH, and D is C_{1-4} alkyl or C_{2-4} alkene; and m is zero or one;

X is selected from $-(CH_2)_rC(O)H$, $-(CH_2)_rC(O)haloalkyl$, $-(CH_2)_rC(O)(CH_2)_rCHN_2$, $-C(CH_2)_r(O)C(CH_2)_r(O)OR_D$, $-(CH_2)_rC(O)(CH_2)_rC(O)NR_D R_E$, $-(CH_2)_rC \equiv N$, $-(CH_2)_rC(OH)(CH_2)_rC(O)U$, $-(CH_2)_rC(OH)CH_2C(O)U$, $-(CH_2)_rC(O)W$ and $-(CH_2)_rC(O)CH_2W$, in which:

R_D and R_E are independently selected from among H, alkyl, preferably lower alkyl, more preferably C_{1-4} alkyl, phenyl, benzyl, and phenethyl; U is $-OR_D$ or $-NR_D R_E$, and W is $-OR_D$, $-SR_D$, and $-NR_D R_E$, or heterocyclic moiety, preferably containing 4-6, more preferably 5 or 6 members in the ring, and preferably one or two heteroatoms, selected from O, S, or N, in the ring, and r is 0-5, preferably 0-3, more preferably 0 or 1, most preferably 0; or

(ii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i),

(iv)(a-c) with R_8 being H or (v)(a-c);

V is OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH;

n is zero; and

R_6 and R_7 are selected so that with the atoms to which each is attached they form a heterocyclic moiety, which:

-139-

5 (a) contains from 3 to 21 members and one or two or more fused rings, each ring containing preferably 3 to 7, more preferably 4 to 6, members, and is preferably morpholino, thiomorpholino, pyrrolidinyl, V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl, or a reduced isoquinoline, preferably 1,2,3,4,tetrahydroisoquinoline;

(b) does not contain adjacent heteroatoms;

10 (c) is unsubstituted or substituted with one or more substituents selected from Y, more preferably from V, and most preferably selected from among OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH; or

(iii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i);

V is as defined in (ii);

15 Q is C(O);

n is one; and

R_6 and R_7 are each independently selected as follows:

20 (a) from carbonyl (C=O), phenyl, a heteroatom, lower alkyl, preferably C_{1-3} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-3} alkyl, and

(b) each is unsubstituted or substituted with Y, preferably with V, and

25 (c) together with the atoms to which they are attached form a cyclic moiety, preferably a 4-6 membered cyclic or 8-12 membered bicyclic moiety, and

(d) R_6 and R_7 are selected with the proviso that when two or more heteroatoms are present there is a carbon atom between the heteroatoms; and

30 (e) the cyclic moiety is preferably succinimide, phthalimide or maleimide; or

-140-

(iv) $R_3, R_4, R_5, R_6, R_7, R_A, R_B, Q, X$ and n are as defined in any of (i)-(iii) or (v)-(vii),

V is as defined in (ii);

R_8 is H; and

5 R_1 and R_2 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

10 (b) R_1 and R_2 are unsubstituted or substituted with Y , preferably with V , and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V -substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

15

(v) $R_1, R_2, R_5, R_6, R_7, R_8, R_A, R_B, X, Q$ and n are as defined in any of (i)-(iv) or (vi)-(vii);

V is as defined in (ii);

20 R_3 and R_4 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

25 (b) is unsubstituted or substituted with Y , preferably with V , and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpho-

-141-

lino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl;

(vi) R_1 , R_2 , R_3 , R_4 , R_7 , R_8 , Q, X and n are as defined in any of (i), (iv)(a-c) with R_6 being H or (v)(a-c);

5 V is as defined in (ii);

R_5 and R_6 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

10 (b) R_6 and R_6 are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

15 (vii) R_1 , R_2 , R_3 , R_4 , R_6 , R_8 , and X are selected as in (i), (iv)(a-c) with R_6 being H or (v)(a-c);

20 V is as defined in (ii);

n is zero; and

R_5 and R_7 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

25 (b) R_5 and R_7 are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered

30

-142-

heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl.

40. A method of treating a patient suffering from a disease
5 characterized by a degradation of the neuronal cytoskeleton resulting from a thrombolytic or hemorrhagic stroke, comprising administering to the patient a therapeutically effective amount of a compound of any of claims 1-32.

41. A method of indicating neurodegenerative disorders
10 characterized by deposition of cerebral amyloid by detecting a decrease in the ratio of α -sAPP to sAPP or in decrease in the amount of α -sAPP in a sample of CSF compared to such ratio or amount in control CSF from individuals who do not have this disorder or compared to predetermined standard ratios and amounts.

15 42. A method of identifying compounds that are effective for treating patients with neurodegenerative disorders characterized by deposition of cerebral amyloid by administering the compound to a subject and detecting an increase in the ratio of α -sAPP to sAPP or an increase in the amount of α -sAPP in a sample of CSF from the subject compared to such ratio or
20 amount in a sample of CSF prior to administering the compound.

43. Use of a compound of any of claims 1-32 for the manufacture of a medicament for treating a neurodegenerative disease that is characterized by the deposition of cerebral amyloid.

44. Use of a compound of any of claims 1-32 for the manufacture
25 of a medicament for treating a disease characterized by a degradation of the neuronal cytoskeleton resulting from a thrombolytic or hemorrhagic stroke.

45. An article of manufacture, comprising packaging material and a compound of any of claims 1-32 contained within the packaging material, wherein the compound is effective for treating a neurodegenerative disease
30 that is characterized by the deposition of cerebral amyloid; and the

-143-

packaging material includes a label that indicates that the compound or salt thereof is used for treating a neurodegenerative disease that is characterized by the deposition of cerebral amyloid.

46. An article of manufacture, comprising packaging material and a compound of any of claims 1-32 contained within the packaging material, wherein the compound is effective for treating a disease characterized by a degradation of the neuronal cytoskeleton resulting from a thrombolytic or hemorrhagic stroke; and the packaging material includes a label that indicates that the compound or salt thereof is used for treating a disease characterized by a degradation of the neuronal cytoskeleton resulting from a thrombolytic or hemorrhagic stroke.

47. A pharmaceutical composition, comprising a therapeutically effective amount of a compound of any of claims 1-32 in a physiologically acceptable carrier.

48. A pharmaceutical composition formulated for single dosage administration, comprising, in a physiologically acceptable carrier, a therapeutically effective amount of a compound of any of claims 1-32.

49. A compound of claim 1, wherein:

- $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_A, R_B, Q, n$ and X are as defined above for formulae (I) and (II), but in which either $R_7-(Q)_n$ in formula (I) and $R_B-CH(R_A)-(Q)_n$ in formulae (II) or X is replaced with a chemical labeling/linker group or a chromophore or a fluorophore useful for identifying and/or isolating proteases.

50. The compound of claim 49, wherein X is replaced with a chromophore or fluorophore selected from among *p*-nitroanilide, 4-methoxy- β -naphthylamide, or 7-amino-4-methylcoumarin.

51. The compound of claim 49, wherein the $R_7-(Q)_n$ moiety and the $(R_B-CH(R_A)-(Q)_n)$ moiety are chemical labeling/linking groups that permit detection and/or isolation of the peptide to which they are coupled.

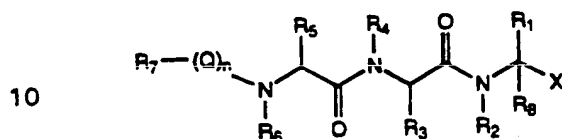
SUBSTITUTE SHEET (RULE 26)

-144-

52. The compound of claim 51, wherein the $R_7-(Q)_n$ moiety and the $(R_8)-CH(R_A)-(Q)_n$ moiety are selected from among biotin, radiolabeled moieties, fluorescein, and primary amines.

53. The compound of claim 52, wherein the amines are 6-amino caproic acid and amino decanoic acid.

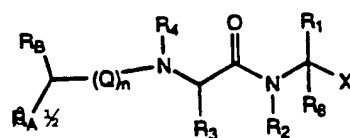
54. Use of a compound of formula (I) or (II):



I

15

20



II

25

30

35

40

-145-

5 or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof, wherein:

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi) or (vii) as follows:

(i) R_1 , R_3 , R_5 , and R_6 , are each independently selected from a side chain of a naturally occurring α -amino acid, H, alkyl, preferably lower (C_{1-6}) alkyl, alkenyl, preferably C_{2-10} alkenyl, alkynyl, preferably C_{2-6} alkynyl, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, Y-substituted aryl, aralkyl, aralkenyl, aralkynyl, and Z-substituted heteroaryl, heteroaralkyl, heteroaralkenyl, in which Y is selected from halogen, lower alkyl, alkoxy, OH, haloalkyl, preferably CF_3 , NO_2 , nitrile, S-alkyl, phenyl, and $-NRR'$, R and R' are independently selected from H or alkyl, preferably lower alkyl, OH and halo-lower alkyl, particularly CF_3 , Z is lower alkyl, preferably C_{1-4} alkyl, or halo lower alkyl, preferably C_{1-4} haloalkyl, more preferably CF_3 ;

20 R_2 , R_4 , R_6 , and R_8 are each independently selected from among H and lower alkyl, preferably C_{1-4} alkyl;

R_7 is selected from among C_{1-6} alkyl, aryl, alkenyl, 9-fluorenyl, aralkyl, aralkenyl, aralkynyl the aryl groups are unsubstituted or are substituted with Z;

25 Q is selected from among $-C(O)-$, $-O-C(O)-$, $-C(O)O-$, $-S(O)_2-$ and $HN-C(O)-$;

n is zero or one;

R_A is $-(T)_m-(D)_m-R_1$ in which T is O or NH, and D is C_{1-4} alkyl or C_{2-4} alkene; and m is zero or one;

30 X is selected from $-(CH_2)_nC(O)H$, $-(CH_2)_nC(O)haloalkyl$, $-(CH_2)_nC(O)(CH_2)_mCHN_2$, $-C(CH_2)_n(O)C(CH_2)_m(O)OR_D$.

SUBSTITUTE SHEET (RULE 26)

-146-

- 5 $-(CH_2)_rC(O)(CH_2)_rC(O)NR_D R_E$, $-(CH_2)_rC \equiv N$, $-(CH_2)_rC(OH)(CH_2)_rC(O)U$,
 $-(CH_2)_rC(OH)CH_2C(O)U$, $-(CH_2)_rC(O)W$ and $-(CH_2)_rC(O)CH_2W$, in which:
 R_D and R_E are independently selected from among H, alkyl, preferably
lower alkyl, more preferably C_{1-4} alkyl, phenyl, benzyl, and phenethyl;
U is $-OR_D$ or $-NR_D R_E$, and W is $-OR_D$, $-SR_D$, and $-NR_D R_E$, or heterocyclic
moiety, preferably containing 4-6, more preferably 5 or 6 members in
the ring, and preferably one or two heteroatoms, selected from O, S,
or N, in the ring, and r is 0-5, preferably 0-3, more preferably 0 or 1,
most preferably 0; or
- 10 (ii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i),
(iv)(a-c) with R_6 being H or (v)(a-c);
V is OH, halogen, lower alkyl, preferably methyl or ethyl or
halogen-substituted lower alkyl, preferably methyl or ethyl, and is
preferably OH;
- 15 n is zero; and
 R_6 and R_7 are selected so that with the atoms to which each is
attached they form a heterocyclic moiety, which:
- 20 (a) contains from 3 to 21 members and one or two or more
fused rings, each ring containing preferably 3 to 7, more
preferably 4 to 6, members, and is preferably morpholino,
thiomorpholino, pyrrolidinyl, V-substituted pyrrolidinyl,
particularly 4-hydroxy pyrrolidinyl, or a reduced isoquinoline,
preferably 1,2,3,4-tetrahydroisoquinoline;
- 25 (b) does not contain adjacent heteroatoms;
- (c) is unsubstituted or substituted with one or more
substituents selected from Y, more preferably from V, and
most preferably selected from among OH, halogen, lower alkyl,
preferably methyl or ethyl or halogen-substituted lower alkyl,
preferably methyl or ethyl, and is preferably OH; or
- 30 (iii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i);

-147-

V is as defined in (iii);

Q is C(O);

n is one; and

R₆ and R₇ are each independently selected as follows:

5 (a) from carbonyl (C=O), phenyl, a heteroatom, lower alkyl, preferably C₁₋₃ alkyl, or lower alkyl linked to a heteroatom, preferably C₁₋₃ alkyl, and

(b) each is unsubstituted or substituted with Y, preferably with V, and

10 (c) together with the atoms to which they are attached form a cyclic moiety, preferably a 4-6 membered cyclic or 8-12 membered bicyclic moiety, and

(d) R₆ and R₇ are selected with the proviso that when two or more heteroatoms are present there is a carbon atom between the heteroatoms; and

15 (e) the cyclic moiety is preferably succinimide, phthalimide or maleimide; or

(iv) R₃, R₄, R₅, R₆, R₇, R₈, Q, X and n are as defined in any of (i)-(iii) or (v)-(vii),

20 V is as defined in (iii);

R₈ is H; and

R₁ and R₂ are each independently selected as follows:

25 (a) from lower alkyl, preferably C₁₋₄ alkyl, or lower alkyl linked to a heteroatom, preferably C₁₋₄ alkyl, or a heteroatom, with the proviso when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R₁ and R₂ are unsubstituted or substituted with Y, preferably with V, and

30 (c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered

SUBSTITUTE SHEET (RULE 26)

-148-

heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

- (v) $R_1, R_2, R_5, R_6, R_7, R_8, R_A, R_B, X, Q$ and n are as defined in any of (i)-(iv) or (vi)-(vii);

V is as defined in (ii);

R_3 and R_4 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) is unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl;

- (vi) $R_1, R_2, R_3, R_4, R_7, R_8, Q, X$ and n are as defined in any of (i), (iv)(a-c) with R_6 being H or (v)(a-c);

V is as defined in (ii);

R_5 and R_6 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_5 and R_6 are unsubstituted or substituted with Y, preferably with V, and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered

SUBSTITUTE SHEET (RULE 23)

-149-

heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

(vii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i),

5 (iv)(a-c) with R_6 being H or (v)(a-c);

V is as defined in (ii);

n is zero; and

R_5 and R_7 are each independently selected as follows:

10 (a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_5 and R_7 are unsubstituted or substituted with Y, preferably with V, and

15 (c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl

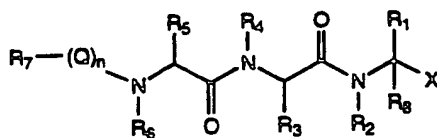
20 for the manufacture of a medicament for treating a neurodegenerative disease that is characterized by the deposition of cerebral amyloid.

55. Use of a compound of any of formula (I) or (II):

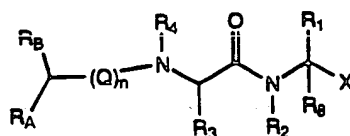
25

30

-150-



I



II

or the hydrates and isosteres, diastereomeric isomers and mixtures thereof, or pharmaceutically acceptable salts thereof, wherein:

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_A , R_B , X , Q and n are selected from among (i), (ii), (iii), (iv), (v), (vi) or (vii) as follows:

(i) R_1 , R_3 , R_5 , and R_8 , are each independently selected from a side chain of a naturally occurring α -amino acid, H, alkyl, preferably lower (C_{1-6}) alkyl, alkenyl, preferably C_{2-10} alkenyl, alkynyl, preferably C_{2-6} alkynyl, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, Y-substituted aryl, aralkyl, aralkenyl, aralkynyl, and Z-substituted heteroaryl, heteroaralkyl, heteroaralkenyl, in which Y is selected from halogen, lower alkyl, alkoxy, OH, haloalkyl, preferably CF_3 , NO_2 , nitrile, S-alkyl, phenyl, and $-\text{NRR}'$, R and R' are independently selected from H or alkyl, preferably low alkyl, OH and halo-lower alkyl, particularly CF_3 , Z is lower alkyl, preferably C_{1-4} alkyl, or halo lower alkyl, preferably C_{1-4} haloalkyl, more preferably CF_3 ;

-151-

R_2 , R_4 , R_6 , and R_8 are each independently selected from among H and lower alkyl, preferably C_{1-4} alkyl;

R_7 is selected from among C_{1-6} alkyl, aryl, alkenyl, 9-fluorenyl, aralkyl, aralkenyl, aralkynyl the aryl groups are unsubstituted or are substituted with Z;

Q is selected from among $-C(O)-$, $-O-C(O)-$, $-C(O)O-$, $-S(O)_2-$ and $HN-C(O)-$;

n is zero or one;

R_A is $-(T)_m-(D)_m-R_1$ in which T is O or NH, and D is C_{1-4} alkyl or C_{2-4} alkene; and m is zero or one;

X is selected from $-(CH_2)_rC(O)H$, $-(CH_2)_rC(O)haloalkyl$, $-(CH_2)_rC(O)(CH_2)_rCHN_2$, $-C(CH_2)_r(O)C(CH_2)_r(O)OR_D$, $-(CH_2)_rC(O)(CH_2)_rC(O)NR_DR_E$, $-(CH_2)_rC\equiv N$, $-(CH_2)_rC(OH)(CH_2)_rC(O)U$, $-(CH_2)_rC(OH)CH_2C(O)U$, $-(CH_2)_rC(O)W$ and $-(CH_2)_rC(O)CH_2W$, in which:

R_D and R_E are independently selected from among H, alkyl, preferably lower alkyl, more preferably C_{1-4} alkyl, phenyl, benzyl, and phenethyl; U is $-OR_D$ or $-NR_DR_E$, and W is $-OR_D$, $-SR_D$, and $-NR_DR_E$, or heterocyclic moiety, preferably containing 4-6, more preferably 5 or 6 members in the ring, and preferably one or two heteroatoms, selected from O, S, or N, in the ring, and r is 0-5, preferably 0-3, more preferably 0 or 1, most preferably 0; or

(ii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i).

(iv)(a-c) with R_8 being H or (v)(a-c);

V is OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH;

n is zero; and

R_6 and R_7 are selected so that with the atoms to which each is attached they form a heterocyclic moiety, which:

-152-

- 5 (a) contains from 3 to 21 members and one or two or more fused rings, each ring containing preferably 3 to 7, more preferably 4 to 6, members, and is preferably morpholino, thiomorpholino, pyrrolidinyl, V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl, or a reduced isoquinoline, preferably 1,2,3,4,tetrahydroisoquinoline;
- (b) does not contain adjacent heteroatoms;
- 10 (c) is unsubstituted or substituted with one or more substituents selected from Y, more preferably from V, and most preferably selected from among OH, halogen, lower alkyl, preferably methyl or ethyl or halogen-substituted lower alkyl, preferably methyl or ethyl, and is preferably OH; or
- (iii) R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are selected as in (i);
- V is as defined in (ii);
- 15 Q is C(O);
- n is one; and
- R_6 and R_7 are each independently selected as follows:
- (a) from carbonyl (C=O), phenyl, a heteroatom, lower alkyl, preferably C_{1-3} alkyl, or lower alkyl linked to a
- 20 heteroatom, preferably C_{1-3} alkyl, and
- (b) each is unsubstituted or substituted with Y, preferably with V, and
- (c) together with the atoms to which they are attached form a cyclic moiety, preferably a 4-6 membered cyclic or 8-12
- 25 membered bicyclic moiety, and
- (d) R_6 and R_7 are selected with the proviso that when two or more heteroatoms are present there is a carbon atom between the heteroatoms; and
- (e) the cyclic moiety is preferably succinimide,
- 30 phthalimide or maleimide; or

SUBSTITUTE SHEET (RULE 26)

-153-

(iv) $R_3, R_4, R_5, R_6, R_7, R_A, R_B, Q, X$ and n are as defined in any of (i)-(iii) or (v)-(vii),

V is as defined in (ii);

R_8 is H; and

R_1 and R_2 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_1 and R_2 are unsubstituted or substituted with Y , preferably with V , and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V -substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

(v) $R_1, R_2, R_5, R_6, R_7, R_8, R_A, R_B, X, Q$ and n are as defined in any of (i)-(iv) or (vi)-(vii);

V is as defined in (ii);

R_3 and R_4 are each independently selected as follows:

(a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) is unsubstituted or substituted with Y , preferably with V , and

(c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpho-

-154-

lino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl;

- (vi) R_1 , R_2 , R_3 , R_4 , R_7 , R_8 , Q, X and n are as defined in any of (i), (iv)(a-c) with R_6 being H or (v)(a-c);

5 V is as defined in (ii);

R_5 and R_6 are each independently selected as follows:

- (a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_5 and R_6 are unsubstituted or substituted with Y, preferably with V, and

- (c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl; or

- (vii) R_1 , R_2 , R_3 , R_4 , R_6 , R_8 , and X are selected as in (i), (iv)(a-c) with R_6 being H or (v)(a-c);

20 V is as defined in (ii);

n is zero; and

R_5 and R_7 are each independently selected as follows:

- (a) from lower alkyl, preferably C_{1-4} alkyl, or lower alkyl linked to a heteroatom, preferably C_{1-4} alkyl, or a heteroatom, with the proviso that when more than one heteroatom is present, there is at least one carbon atom between each heteroatom, and

(b) R_5 and R_7 are unsubstituted or substituted with Y, preferably with V, and

- (c) together with the atoms to which they are attached form a heterocyclic moiety, preferably a 4-6 membered

-155-

heterocyclic moiety, that is preferably morpholino, thiomorpholino, pyrrolidinyl, or V-substituted pyrrolidinyl, particularly 4-hydroxy pyrrolidinyl.

- 5 for the manufacture of a medicament for treating a disease characterized by a degradation of the neuronal cytoskeleton resulting from a thrombolytic or hemorrhagic stroke.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 96/00359

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/06 C07K5/08 C07K5/02 C07C237/22 A61K38/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 457 195 (FUJISAWA) 21 November 1991 see page 31, line 32 - line 45; claims; examples ---	1,21
X	EP,A,0 019 589 (PENTAPHARM AG) 26 November 1980 see claims; examples ---	1,21, 48-51
X	EP,A,0 460 679 (BANYU PHARMACEUTICAL CO.) 11 December 1991 see claims; examples 13-17 ---	1,21,48, 49
X	EP,A,0 266 950 (PFIZER) 11 May 1988 see claims; examples 1,4,5,38,134 ---	1,11,21, 48,49
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- 'A' document member of the same patent family

Date of the actual completion of the international search

28 May 1996

Date of mailing of the international search report

07.06.96

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 360-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 360-3016

Authorized officer

Helps, I

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/00359

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,4 472 305 (HANSEN ET. AL.) 18 September 1984 see example 8 ---	1,21
X	EP,A,0 528 629 (BEND RESEARCH) 24 February 1993 see table 1, compounds IIIk, IIIl, IIIlt, claims ---	1,11,14, 15,21, 48,49
A	EP,A,0 504 938 (SUNTORY LTD.) 23 September 1992 see page 1 - page 3; table 1 ---	1,33,48, 56
X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 36, no. 17, 20 August 1993, WASHINGTON DC, US, pages 2431-47, XP002003956 D. W. PATEL ET. AL. : "Activated Ketone Based Inhibitors of Human Renin." see page 2433, Compound 13, page 2434, Compound 20 and page 2436, Compound 33 ---	1,11,15, 21,48,49
X	JOURNAL OF ENZYME INHIBITION, vol. 3, no. 3, 1990, NEW YORK, US, pages 195-200, XP002003957 T. SASAKI ET. AL.: "Inhibitory Effect of Di-and Tripeptidyl Aldehydes on Calpains and Cathepsins." see page 196, paragraph 3 - page 197, paragraph 1 ---	1,21
A	FEBS LETTERS, vol. 319, no. 3, March 1993, AMSTERDAM, NL, pages 253-6, XP002003958 E. SARRUBI ET. AL.: "Peptide aldehydes as inhibitors of HIV Protease" see whole document ---	1,33,48, 56
A	BIOCHEMISTRY, vol. 31, no. 39, 6 October 1992, pages 9421-8, XP002003959 A. VINITSKY ET. AL. : "Inhibition of the Chymotrypsin-like Activity of the Pituitary Multicatalytic Proteinase Complex." see whole document ---	1,33,48, 56

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/00359

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 114, no. 19, 13 May 1991 Columbus, Ohio, US; abstract no. 186081c, T. ITO: "Preparation of N-benzylloxycarbonyl tripeptide aldehydes for treatment of senile dementia." page 850; column 1; XP002003961 see abstract & JP,A,02 268 196 (UBE INDUSTRIES, LTD.) ----	1,33-36, 44,48,49
A	EP,A,0 569 777 (MILES, INC.) 18 November 1993 see page 1 - page 8; claims ----	1,33,42
X	NEUROSCIENCE LETTERS, vol. 201, no. 1, January 1995, SHANNON, IE. pages 29-32, XP002003960 H. W. KLAFFKI ET. AL.: "Calpain inhibitor I decreases beta-A4 secretion from human embryonal kidney cells expressing beta-amyloid precursor protein carrying the APP670/671 double mutation." see whole document -----	1,33-49, 55,56

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/00359

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0457195	21-11-91	AU-B- 7644691	14-11-91
		CA-A- 2042442	15-11-91
		CN-A- 1057269	25-12-91
		JP-A- 4244097	01-09-92
		US-A- 5430022	04-07-95
		US-A- 5284828	08-02-94
EP-A-0019589	26-11-80	AT-T- 5254	15-11-83
		AU-B- 547872	07-11-85
		AU-B- 5990480	03-12-80
		CA-A- 1161431	31-01-84
		WO-A- 8002559	27-11-80
		JP-C- 1616944	30-08-91
		JP-B- 2044839	05-10-90
		JP-A- 62294695	22-12-87
		JP-C- 1616945	30-08-91
		JP-B- 2044840	05-10-90
		JP-A- 62294696	22-12-87
		JP-C- 1616946	30-08-91
		JP-B- 2044518	04-10-90
		JP-A- 62296899	24-12-87
		JP-C- 1450372	11-07-88
		JP-A- 57002253	07-01-82
		JP-B- 62057197	30-11-87
EP-A-0460679	11-12-91	AU-B- 632695	07-01-93
		AU-B- 7818291	12-12-91
		JP-A- 5178891	20-07-93
		US-A- 5470833	28-11-95
		US-A- 5444152	22-08-95
		US-A- 5496928	05-03-96
EP-A-0266950	11-05-88	AU-B- 585180	08-06-89
		AU-B- 8054187	05-05-88
		CA-A- 1310793	24-11-92
		CN-B- 1027271	04-01-95
		DE-D- 3788626	10-02-94
		DE-T- 3788626	28-04-94
		ES-T- 2061512	16-12-94
		IE-B- 61087	21-09-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/00359

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0266950		JP-B- 7108901 JP-A- 7173134 JP-A- 63183551 SU-A- 1706391 US-A- 4814342 US-A- 4935405 US-A- 5034376	22-11-95 11-07-95 28-07-88 15-01-92 21-03-89 19-06-90 23-07-91
US-A-4472305	18-09-84	NONE	
EP-A-528629	24-02-93	CA-A- 2075771 JP-A- 5292992	13-02-93 09-11-93
EP-A-504938	23-09-92	JP-A- 5155764 JP-A- 5178758	22-06-93 20-07-93
EP-A-0569777	18-11-93	AU-B- 3711093 CA-A- 2095888 WO-A- 9513084 ZA-A- 9303249 AU-B- 5726494 CA-A- 2151927 EP-A- 0694076 WO-A- 9413319	18-11-93 12-11-93 18-05-95 11-03-94 04-07-94 23-06-94 31-01-96 23-06-94